```
FILE 'HOME' ENTERED AT 16:19:15 ON 18 SEP 2002

=> fil reg
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILE 'REGISTRY' ENTERED AT 16:19:22 ON 18 SEP 2002
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STRUCTURE FILE UPDATES:   17 SEP 2002  HIGHEST RN 452274-20-3
DICTIONARY FILE UPDATES:  17 SEP 2002  HIGHEST RN 452274-20-3

TSCA INFORMATION NOW CURRENT THROUGH MAY 20, 2002

  Please note that search-term pricing does apply when
  conducting SmartSELECT searches.

Crossover limits have been increased.  See HELP CROSSOVER for details.

Experimental and calculated property data are now available.  See HELP
PROPERTIES for more information.  See STNote 27, Searching Properties
in the CAS Registry File, for complete details:
http://www.cas.org/ONLINE/STN/STNOTES/stnotes27.pdf

=> .:..Testing the current file.... screen

ENTER SCREEN EXPRESSION OR (END):end


=>
Uploading C:\STNEXP4\QUERIES\reissue.str

L1      STRUCTURE UPLOADED

=> que L1

L2   QUE L1

=> d l2
L2 HAS NO ANSWERS
L1              STR

* STRUCTURE DIAGRAM TOO LARGE FOR DISPLAY -  AVAILABLE VIA OFFLINE PRINT *

Structure attributes must be viewed using STN Express query preparation.
L2              QUE  ABB=ON  PLU=ON  L1


=> s l1
SAMPLE SEARCH INITIATED 16:19:55 FILE 'REGISTRY'
SAMPLE SCREEN SEARCH COMPLETED -      1 TO ITERATE

100.0% PROCESSED       1 ITERATIONS                              0 ANSWERS
SEARCH TIME: 00.00.01
```

```
FULL FILE PROJECTIONS:  ONLINE  **COMPLETE**
                        BATCH   **COMPLETE**
PROJECTED ITERATIONS:            1 TO       80
PROJECTED ANSWERS:               0 TO        0

L3            0 SEA SSS SAM L1

=> s l1 full
FULL SEARCH INITIATED 16:20:03 FILE 'REGISTRY'
FULL SCREEN SEARCH COMPLETED -     63 TO ITERATE

100.0% PROCESSED      63 ITERATIONS                                0 ANSWERS
SEARCH TIME: 00.00.04

L4            0 SEA SSS FUL L1

=> d l1
L1 HAS NO ANSWERS
L1              STR

* STRUCTURE DIAGRAM TOO LARGE FOR DISPLAY -  AVAILABLE VIA OFFLINE PRINT *

Structure attributes must be viewed using STN Express query preparation.


=> s l2
SAMPLE SEARCH INITIATED 16:20:48 FILE 'REGISTRY'
SAMPLE SCREEN SEARCH COMPLETED -      1 TO ITERATE

100.0% PROCESSED       1 ITERATIONS                                0 ANSWERS
SEARCH TIME: 00.00.01

FULL FILE PROJECTIONS:  ONLINE  **COMPLETE**
                        BATCH   **COMPLETE**
PROJECTED ITERATIONS:            1 TO       80
PROJECTED ANSWERS:               0 TO        0

L5            0 SEA SSS SAM L1

=> s l2 full
FULL SEARCH INITIATED 16:20:56 FILE 'REGISTRY'
FULL SCREEN SEARCH COMPLETED -     63 TO ITERATE

100.0% PROCESSED      63 ITERATIONS                                0 ANSWERS
SEARCH TIME: 00.00.02

L6            0 SEA SSS FUL L1

=> fil marpat
COST IN U.S. DOLLARS                              SINCE FILE      TOTAL
                                                     ENTRY    SESSION
FULL ESTIMATED COST                                 280.94     281.15

FILE 'MARPAT' ENTERED AT 16:21:25 ON 18 SEP 2002
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FILE CONTENT: 1988-PRESENT (VOL 104 ISS 15-VOL 137 ISS 10)(20020906/ED)
```

MOST RECENT CITATIONS FOR PATENTS FROM FIVE MAJOR ISSUING AGENCIES
(COVERAGE TO THESE DATES IS NOT COMPLETE):

US 200209125 11 JUL 2002
DE 20202609 25 JUL 2002
EP 1231213 14 AUG 2002
JP 200222667 14 AUG 2002
WO 200206302 15 AUG 2002

Structure search limits have been raised. See HELP SLIMIT for the new, higher limits.

=> s l1
SAMPLE SEARCH INITIATED 16:21:32 FILE 'MARPAT'
SAMPLE SCREEN SEARCH COMPLETED - 1505 TO ITERATE

66.4% PROCESSED 1000 ITERATIONS 0 ANSWERS
INCOMPLETE SEARCH (SYSTEM LIMIT EXCEEDED)
SEARCH TIME: 00.00.08

FULL FILE PROJECTIONS: ONLINE **COMPLETE**
BATCH **COMPLETE**
PROJECTED ITERATIONS: 27977 TO 32223
PROJECTED ANSWERS: 0 TO 0

L7 0 SEA SSS SAM L1

=> s l1 full
FULL SEARCH INITIATED 16:21:46 FILE 'MARPAT'
FULL SCREEN SEARCH COMPLETED - 30333 TO ITERATE

17.8% PROCESSED 5392 ITERATIONS 0 ANSWERS

47.1% PROCESSED 14273 ITERATIONS (1 INCOMPLETE) 1 ANSWERS

67.0% PROCESSED 20326 ITERATIONS (2 INCOMPLETE) 3 ANSWERS

78.3% PROCESSED 23753 ITERATIONS (2 INCOMPLETE) 3 ANSWERS

91.9% PROCESSED 27873 ITERATIONS (4 INCOMPLETE) 5 ANSWERS

97.8% PROCESSED 29656 ITERATIONS (4 INCOMPLETE) 5 ANSWERS

99.8% PROCESSED 30276 ITERATIONS (4 INCOMPLETE) 5 ANSWERS

100.0% PROCESSED 30333 ITERATIONS (4 INCOMPLETE) 5 ANSWERS
SEARCH TIME: 00.02.15

L8 5 SEA SSS FUL L1

=> d l8 1-5

L8 ANSWER 1 OF 5 MARPAT COPYRIGHT 2002 ACS
AN 136:304094 MARPAT
TI Insulin receptor activators for the treatment of metabolic disorders in humans resulting from treatment of HIV infection with HIV protease inhibitors
IN Manchem, Prasad V. V. S. V.; Lum, Robert T.; Schow, Steven R.
PA Telik, Inc., USA

SO PCT Int. Appl., 48 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002030514	A2	20020418	WO 2001-US42733	20011010
	W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	FR 2814953	A1	20020412	FR 2001-13040	20011010
	US 2002061927	A1	20020523	US 2001-977059	20011011
PRAI	US 2000-239636P	20001011			

L8 ANSWER 2 OF 5 MARPAT COPYRIGHT 2002 ACS
(ALL HITs ARE ITERATION INCOMPLETES)
AN 133:89443 MARPAT
TI Quinolinecarboxamides as antiviral agents, especially against viruses of the herpes family
IN Turner, Steven Ronald; Strohbach, Joseph Walter; Thaisrivongs, Suvit; Vaillancourt, Valerie A.; Schnute, Mark E.; Tucker, John Alan
PA Pharmacia & Upjohn Company, USA
SO PCT Int. Appl., 219 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000040561	A1	20000713	WO 1999-US27960	19991222
	W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	US 6248739	B1	20010619	US 1999-466712	19991217
	EP 1140850	A1	20011010	EP 1999-967145	19991222
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	NO 2001003383	A	20010907	NO 2001-3383	20010706
PRAI	US 1999-115301P	19990108			
	US 1999-140610P	19990623			
	WO 1999-US27960	19991222			

RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 3 OF 5 MARPAT COPYRIGHT 2002 ACS
(ALL HITs ARE ITERATION INCOMPLETES)

AN 125:53049 MARPAT
TI Chemical process for promoting the proliferation of animal cells
IN Renner, Wolfgang A.; Eppenberger, Hans M.; Bailey, James Edwin
PA Switz.
SO PCT Int. Appl., 30 pp.
CODEN: PIXXD2
DT Patent
LA German
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9607730	A2	19960314	WO 1995-CH191	19950905
	WO 9607730	A3	19960418		
	W: US				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	EP 733100	A1	19960925	EP 1995-928931	19950905
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
PRAI	CH 1994-2763		19940909		
	WO 1995-CH191		19950905		

L8 ANSWER 4 OF 5 MARPAT COPYRIGHT 2002 ACS
(ALL HITs ARE ITERATION INCOMPLETES)
AN 118:233893 MARPAT
TI New 6(7)-amino-substituted-5,8-quinolinediones to combat endoparasites
IN Jeschke, Peter; Lindner, Werner; Mueller, Nikolaus; Harder, Achim; Mencke,
Norbert
PA Bayer A.-G., Germany
SO Eur. Pat. Appl., 37 pp.
CODEN: EPXXDW
DT Patent
LA German
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 519290	A1	19921223	EP 1992-109623	19920609
	R: BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE				
	DE 4120477	A1	19921224	DE 1991-4120477	19910621
	AU 9217393	A1	19921224	AU 1992-17393	19920603
	JP 05221996	A2	19930831	JP 1992-178934	19920615
	CA 2071566	AA	19921222	CA 1992-2071566	19920618
	ZA 9204516	A	19930331	ZA 1992-4516	19920619
PRAI	DE 1991-4120477		19910621		

L8 ANSWER 5 OF 5 MARPAT COPYRIGHT 2002 ACS
(ALL HITs ARE ITERATION INCOMPLETES)
AN 116:13416 MARPAT
TI Pressure- and heat-sensitive recording materials with good sensitivity, storability and image stability
IN Sano, Masajiro; Takashima, Masanobu; Satomura, Masato
PA Fuji Photo Film Co., Ltd., Japan
SO Jpn. Kokai Tokkyo Koho, 11 pp.
CODEN: JKXXAF
DT Patent
LA Japanese
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 03142277	A2	19910618	JP 1989-282319	19891030

Welcome to STN International! Enter x:x

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NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
NEWS 2	Apr 08	"Ask CAS" for self-help around the clock
NEWS 3	Apr 09	BEILSTEIN: Reload and Implementation of a New Subject Area
NEWS 4	Apr 09	ZDB will be removed from STN
NEWS 5	Apr 19	US Patent Applications available in IFICDB, IFIPAT, and IFIUDB
NEWS 6	Apr 22	Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
NEWS 7	Apr 22	BIOSIS Gene Names now available in TOXCENTER
NEWS 8	Apr 22	Federal Research in Progress (FEDRIP) now available
NEWS 9	Jun 03	New e-mail delivery for search results now available
NEWS 10	Jun 10	MEDLINE Reload
NEWS 11	Jun 10	PCTFULL has been reloaded
NEWS 12	Jul 02	FOREGE no longer contains STANDARDS file segment
NEWS 13	Jul 22	USAN to be reloaded July 28, 2002; saved answer sets no longer valid
NEWS 14	Jul 29	Enhanced polymer searching in REGISTRY
NEWS 15	Jul 30	NETFIRST to be removed from STN
NEWS 16	Aug 08	CANCERLIT reload
NEWS 17	Aug 08	PHARMAMarketLetter(PHARMAML) - new on STN
NEWS 18	Aug 08	NTIS has been reloaded and enhanced
NEWS 19	Aug 19	Aquatic Toxicity Information Retrieval (AQUIRE) now available on STN
NEWS 20	Aug 19	IFIPAT, IFICDB, and IFIUDB have been reloaded
NEWS 21	Aug 19	The MEDLINE file segment of TOXCENTER has been reloaded
NEWS 22	Aug 26	Sequence searching in REGISTRY enhanced
NEWS 23	Sep 03	JAPIO has been reloaded and enhanced
NEWS 24	Sep 16	Experimental properties added to the REGISTRY file
NEWS 25	Sep 16	Indexing added to some pre-1967 records in CA/CAPLUS
NEWS 26	Sep 16	CA Section Thesaurus available in CAPLUS and CA

NEWS EXPRESS	February 1 CURRENT WINDOWS VERSION IS V6.0d, CURRENT MACINTOSH VERSION IS V6.0a(ENG) AND V6.0Ja(JP), AND CURRENT DISCOVER FILE IS DATED 05 FEBRUARY 2002
NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS INTER	General Internet Information
NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
NEWS WWW	CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that specific topic.

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* * * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 11:37:37 ON 19 SEP 2002

=> fil reg

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.21	0.21

FILE 'REGISTRY' ENTERED AT 11:37:45 ON 19 SEP 2002
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Property values tagged with IC are from the ZIC/VINITI data file provided by InfoChem.

STRUCTURE FILE UPDATES: 18 SEP 2002 HIGHEST RN 452896-77-4
DICTIONARY FILE UPDATES: 18 SEP 2002 HIGHEST RN 452896-77-4

TSCA INFORMATION NOW CURRENT THROUGH MAY 20, 2002

Please note that search-term pricing does apply when conducting SmartSELECT searches.

Crossover limits have been increased. See HELP CROSSOVER for details.

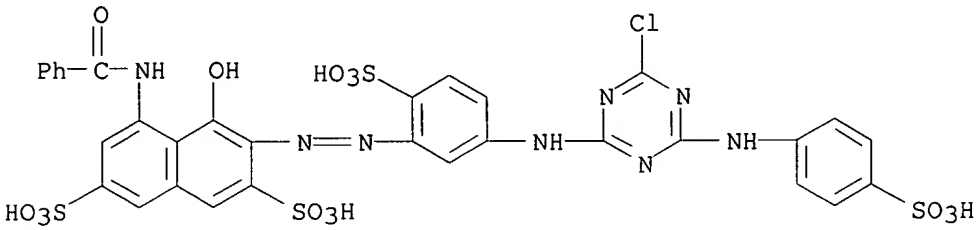
Experimental and calculated property data are now available. See HELP PROPERTIES for more information. See STNote 27, Searching Properties in the CAS Registry File, for complete details:
http://www.cas.org/ONLINE/STN/STNOTES/stnotes27.pdf

=> s TER 12/cn
L1 1 TER 12/CN

=> d

L1 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2002 ACS
RN 17681-50-4 REGISTRY
CN 2,7-Naphthalenedisulfonic acid, 5-(benzoylamino)-3-[[5-[[4-chloro-6-[(4-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-2-sulfophenyl]azo]-4-hydroxy-, tetrasodium salt (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN C.I. Reactive Red 4, tetrasodium salt (8CI)
CN Cibacron Brilliant Red 3B (6CI)
OTHER NAMES:
CN C.I. 18105
CN C.I. Reactive Red 4
CN Cibacron Brilliant Red 3B-A
CN Cibacron Red 3BA
CN Procion Brilliant Red H 7B
CN Procion Brilliant Red H 7BS
CN Reactive Red 4

CN **TER 12**
MF C32 H23 Cl N8 O14 S4 . 4 Na
LC STN Files: BEILSTEIN*, BIOBUSINESS, BIOSIS, CA, CAOLD, CAPLUS, CHEMCATS,
CHEMLIST, CSCHEM, IFICDB, IFIPAT, IFIUDB, MSDS-OHS, TOXCENTER, USPATFULL
(*File contains numerically searchable property data)
Other Sources: DSL**, EINECS**, TSCA**
(**Enter CHEMLIST File for up-to-date regulatory information)
CRN (16480-43-6)



●4 Na

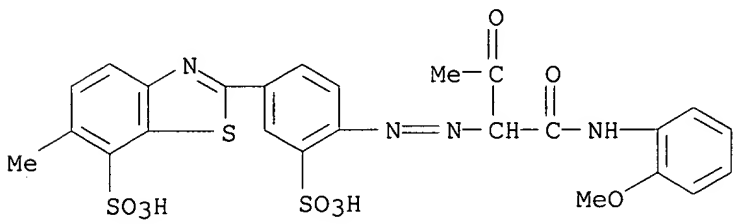
102 REFERENCES IN FILE CA (1967 TO DATE)
13 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
103 REFERENCES IN FILE CAPLUS (1967 TO DATE)
2 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

=> S TER 3938/cn
L2 1 TER 3938/CN

=> d

L2 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2002 ACS
RN 10190-68-8 REGISTRY
CN 7-Benzothiazolesulfonic acid,
2-[4-[[1-[[(2-methoxyphenyl)amino]carbonyl]-
2-oxopropyl]azo]-3-sulfophenyl]-6-methyl-, disodium salt (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN C.I. Direct Yellow 27, disodium salt (8CI)
CN Solar Flavine 5G (6CI)
OTHER NAMES:
CN Benzo Viscose Yellow 5GL
CN C.I. 13950
CN C.I. Direct Yellow 27
CN Chlorantine Fast Yellow 7GL
CN Diazol Light Yellow 7JL
CN Diphenyl Fast Brilliant Yellow 8GL
CN Direct Yellow 27
CN Fastusol Yellow L 5GA
CN Fenaluz Yellow 4G
CN Helion Yellow 5G

CN Hispaluz Yellow 5G
CN Orbantin Yellow 5G
CN Pyrazol Fast Flavine 5G
CN Sirius Supra Yellow 5G
CN Solamine Fast Yellow 5G
CN Solamine Light Yellow 5G
CN Solantine Yellow 8GL
CN Solex Canary Yellow 5G
CN Solius Light Yellow 5G
CN **TER 3938**
CN Tertrodirect Fast Yellow 8G
CN Tetramine Fast Yellow Extra-greenish
DR 98113-29-2, 51052-88-1
MF C25 H22 N4 O9 S3 . 2 Na
LC STN Files: CA, CAOLD, CAPLUS, CHEMCATS, CHEMLIST, CSCHEM, TOXCENTER, USPAT2, USPATFULL
Other Sources: DSL**, EINECS**, TSCA**
(**Enter CHEMLIST File for up-to-date regulatory information)



25 REFERENCES IN FILE CA (1967 TO DATE)
25 REFERENCES IN FILE CAPLUS (1967 TO DATE)
1 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

=> s TER 3935/cn
L3 0 TER 3935/CN

=> TER 16998/cn
TER IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s TER 16998/cn
L4 1 TER 16998/CN

=> d

L4 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2002 ACS
RN 210978-64-6 REGISTRY
CN 2-Naphthalenesulfonic acid,
4-hydroxy-6-[[[[5-hydroxy-7-sulfo-6-[[2-sulfo-
4-[(4-sulfophenyl)azo]phenyl]azo]-2-naphthalenyl]amino]carbonyl]amino]-1-
[[2-sulfo-4-[(4-sulfophenyl)azo]phenyl]azo]-, hexasodium salt (9CI) (CA

INDEX NAME)
OTHER NAMES:
CN **TER 16998**
CN TLK 16998
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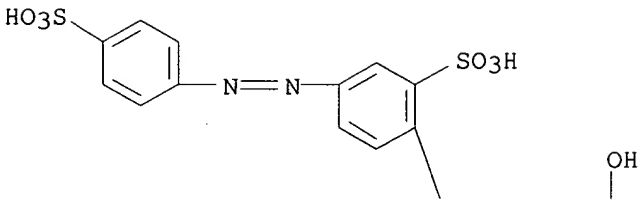
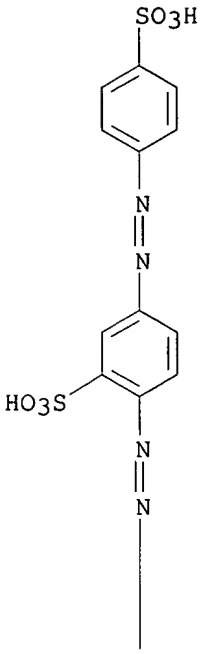
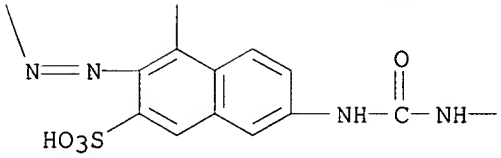
SR CA
LC STN Files: BIOSIS, CA, CAPLUS, USPATFULL



PAGE 1-B

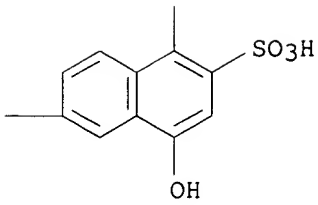


PAGE 2-A



●6 Na

PAGE 2-B



3 REFERENCES IN FILE CA (1967 TO DATE)
3 REFERENCES IN FILE CAPLUS (1967 TO DATE)

```
=>
=> fil embase caplus uspatfull biosis medline
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                      23.40      23.61

FILE 'EMBASE' ENTERED AT 11:41:41 ON 19 SEP 2002
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CA INDEXING COPYRIGHT (C) 2002 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'BIOSIS' ENTERED AT 11:41:41 ON 19 SEP 2002
COPYRIGHT (C) 2002 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'MEDLINE' ENTERED AT 11:41:41 ON 19 SEP 2002

=> s kinae and insulin
L5          15 KINAE AND INSULIN

=> s kinase and insulin
L6       46497 KINASE AND INSULIN

=> s 17681-50-4/rn or 10190-68-8/rn or 210978-64-6/rn
'RN' IS NOT A VALID FIELD CODE
'RN' IS NOT A VALID FIELD CODE
'RN' IS NOT A VALID FIELD CODE
L7         170 17681-50-4/RN OR 10190-68-8/RN OR 210978-64-6/RN

=> s l6 and l7
L8           7 L6 AND L7

=> dup rem l8
PROCESSING COMPLETED FOR L8
L9           7 DUP REM L8 (0 DUPLICATES REMOVED)

=> d l9 1-7 ab bib kwic

L9   ANSWER 1 OF 7  USPATFULL
AB     Modulation of the activity of the insulin receptor,
       enhancement of glucose uptake by cells, and other effects significant
in
       the control and management of diabetes are accomplished using compounds
       of the formula   ##STR1##

       wherein each A is independently a proton-accepting substituent;

       each R is independently a noninterfering substituent;

       m is 0 or 1;

       n is 0, 1, or 2; and
```

each linker is independently an isostere of --N.dbd.N-- or of --NHCO--. Compounds in the genus of Formula (1) can also be used for structure activity studies to identify features responsible for the relevant activities.

AN 2002:27519 USPATFULL

TI Nonpeptide **insulin** receptor agonists

IN Sportsman, Richard, Palo Alto, CA, UNITED STATES
Villar, Hugo O., Newark, CA, UNITED STATES
Kauvar, Lawrence M., San Francisco, CA, UNITED STATES
Satyam, Apparao, Fremont, CA, UNITED STATES

PI US 2002016367 A1 20020207

AI US 2001-961179 A1 20010921 (9)

RLI Division of Ser. No. US 1997-916088, filed on 21 Aug 1997, PENDING
Continuation of Ser. No. US 1997-785855, filed on 20 Jan 1997, GRANTED, Pat. No. US 6073168

DT Utility

FS APPLICATION

LREP HELLER EHRMAN WHITE & MCAULIFFE LLP, 275 MIDDLEFIELD ROAD, MENLO PARK, CA, 94025-3506

CLMN Number of Claims: 43

ECL Exemplary Claim: 1

DRWN 9 Drawing Page(s)

LN.CNT 827

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

TI Nonpeptide **insulin** receptor agonists

AB Modulation of the activity of the **insulin** receptor, enhancement of glucose uptake by cells, and other effects significant in the control and management of diabetes are accomplished. . .

SUMM . . . for peptide ligands that activate hormone receptors. More specifically, it concerns simple nonpeptide compounds that behave as agonists for the **insulin** receptor and enhance the effect of **insulin** on this receptor.

SUMM . . . receptors specific for them so that the activity of the hormone is felt on designated cells exhibiting these receptors. The **insulin** receptor is present on virtually all cells and at high concentrations on the cells of the liver, skeletal muscles, and adipose tissue. Stimulation of the **insulin** receptor with **insulin** is an essential element in carbohydrate metabolism and storage.

SUMM [0003] Diabetics either lack sufficient endogenous secretion of the **insulin** hormone (Type I) or have an **insulin** receptor-mediated signalling pathway that is to some degree resistant to endogenous or exogenous **insulin**, either through primary or post-translational structural changes, reduced numbers or poor coupling among signaling components (Type II). All Type I diabetics, and many Type II subjects as well, must utilize injection to obtain enhanced activity of the extant **insulin** receptors, since endogenous **insulin** can at present be replaced only with an alternative supply of **insulin** itself, previously isolated from native sources, and now recombinantly produced. While the recombinant production of **insulin** permits a less immunogenic form to be provided and assures a reliable supply of needed quantities, the necessity to administer. . . of peptides and proteins in the digestive tract. It has long been the goal to substitute for peptide ligands, including **insulin**, small molecules which are not digested and can be absorbed directly into the bloodstream. However, to date, nonpeptide substances which can exert the effect of

insulin on its receptor have eluded discovery

SUMM . . . a peptide hormone. The ability of certain thiazolidinediones such as pioglitazone to enhance adipocyte differentiation by stimulating the effect of **insulin** has been described by, for example, Kletzien, R. F. et al. J Mol Pharmacol (1992) 41:393-398. These represent a class of potential antidiabetic compounds that act at an unknown site downstream from the **insulin** receptor itself and enhance the response of target tissues to **insulin**. Kobayashi, M. Diabetes (1992) 41:476-483. It is now known that most of the thiazolidinediones bind to PPAR.gamma. thus triggering certain nuclear events that may result in enhanced sensitivity of the target cells to **insulin**. However, the complete mechanism is still unresolved.

SUMM . . . that several aryl di- or polysulfonate compounds which share certain common structural features are able to effect stimulation of the **insulin** receptor to activate the autophosphorylation activity required for signal transduction. The availability of these compounds permits construction of assays and. . . comparative procedures for evaluating additional candidate compounds as well as the design and synthesis of therapeutics for primary treatment of **insulin** resistance and diabetics with the appropriate structural features

SUMM . . . compounds, whose synthesis is straightforward, in order to conduct assays for the ability of candidate small molecules to activate the **insulin** receptor and to design these candidates. The method of identifying a primary member of this group, TER12 and of obtaining the remaining members is described below. These small molecules represent the first instance of direct agonist activity on the **insulin** receptor by a nonpeptide. Compounds identified in this way are useful in the control and management of diabetes in suitable.

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SUMM [0009] Thus, the invention is directed to methods to modulate the **kinase** activity of the **insulin** receptor or the **kinase** portion thereof; to potentiate **insulin** activation of the **insulin** receptor; to potentiate glucose uptake stimulation by **insulin**; to lower blood glucose; and to stimulate glucose uptake per se in cells by use of compounds having the formula. . .

SUMM [0016] In another aspect, the invention is directed to a method to screen candidate compounds for ability to activate the **insulin** receptor. The method comprises first obtaining a fingerprint of each candidate with respect to a reference panel and obtaining a fingerprint of TER12, TER3938, TER3935, TER16998, TER17003 or other compound shown to activate the **kinase** activity of the **insulin** receptor with respect to the same reference panel. Then the fingerprint of each candidate is compared with that of any. . .

SUMM . . . In another aspect, the invention relates to a method to design and synthesize a molecule that exhibits agonist activity or **insulin** agonist stimulating activity with respect to the **insulin** receptor. This method comprises assessing an activator identified as above for structural features which correlate with said activities. Structural features. . .

SUMM [0019] In still another aspect, the invention provides an alternative method to identify a candidate compound which will activate the **insulin** receptor. This method comprises contacting a sample containing at least the **kinase** portion of the **insulin** receptor with an activator identified by any of the foregoing methods in

the presence and absence of said candidate.

DRWD [0021] FIG. 1 shows a schematic diagram of the **insulin** receptor and its activation by **insulin**.

DRWD [0022] FIGS. 2A-2F show the structures of several compounds relevant to the invention which activate the **insulin** receptor.

DRWD [0030] FIG. 4 shows the effect of Component A on **insulin** -induced uptake of glucose by adipocytes.

DRWD [0032] FIG. 6 shows the effect of TER16998, alone and in combination with **insulin**, on autophosphorylation of the IR receptor.

DRWD [0033] FIG. 7 shows the effect of TER16998 on **insulin**-induced glucose uptake in adipocytes.

DETD [0036] The structure of the **insulin** receptor and some aspects of its mode of action as currently understood, are illustrated in FIG. 1. The receptor consists. . . .beta. chains contain a cross-membrane domain, .alpha. the a portions are in the extracellular domain and accommodate the binding of **insulin**. The illustration in FIG. 1 shows **insulin** bound to the receptor. The .beta. subunits contain a tyrosine **kinase** activity, shown as the white inserts into the subunits and the **kinase** of one .beta. subunit effects the phosphorylation of the complementary .beta. subunit as shown, the receptor illustrated in FIG. 1. . . in its activated form when the tyrosine residues (Y) are phosphorylated. The .beta. subunits also contain ATP binding sites. The **insulin**-stimulated phosphorylation of the receptor itself is required for subsequent activity and thus demonstration of the ability of a compound to. . .

DETD . . . to methods to regulate and manage subjects with diabetes by virtue of administering compounds which affect the activity of the **insulin** receptor. Without intending to be bound by any theory, it is believed that the compounds useful in the methods of the invention act directly on the **kinase** function of the receptor and do not necessarily compete with **insulin** for binding at the **insulin**-binding site, nor do they effect activation of the receptor by a mechanism similar to that exhibited by **insulin** The compounds of the invention are able directly to activate the **kinase** of the receptor to autophosphorylate, to potentiate the effect of **insulin** on the receptor, to activate the **kinase** function of the receptor in phosphorylating exogenous substrates, to effect the increased uptake of glucose by adipocytes and **insulin** receptor-bearing cells in general, and to lower blood glucose level in diabetic subjects.

DETD . . . comprises, in a preferred embodiment, contacting each member of a set of maximally diverse candidate compounds with said receptor or **kinase** portion thereof; detecting the presence or absence of tyrosine phosphate on the receptor or **kinase** portion contacted with each set member; and identifying as a successful candidate at least one member of the set wherein an increased amount of tyrosine phosphate is detected in the receptor or **kinase** with which it was contacted, relative to untreated receptor.

DETD [0055] In addition, once a compound with at least moderate ability to activate the **kinase** activity of **insulin** receptor has been identified, additional compounds can be identified by comparing the properties of the candidates with those of compounds. . . This is described in U.S. Pat. No. 5,587,293, incorporated herein by reference. Further, analysis of compounds shown to activate the **insulin** receptor **kinase** using standard structure activity analysis

will result in additional compounds which behave as activators. Compounds identified as activators of the. . .

DETD [0056] The three primary methods of identification of compounds with the desired IR **kinase** modulating activity are illustrated below.

DETD [0057] The activator compounds are able to stimulate the phosphorylation catalyzed by IR **kinase** alone, i.e., to behave as agonists with respect to the receptor and/or are able to enhance the ability of **insulin** to effect phosphorylation of the receptor. Either of these effects can be considered an activation of the **insulin** receptor. Thus, by "activating" the **insulin** receptor is meant either the ability to behave as an agonist or the ability to enhance the stimulation by **insulin** or other agonists of the receptor activity. Both of these effects can be evidenced by autophosphorylation of the receptor.

DETD [0058] The compounds of the invention evidently do not interact with the receptor at the native **insulin** binding site, but rather at a site located on the **kinase** portion of the receptor. Thus, these compounds define a newly discovered activation site for this receptor. This permits not only. . . with the same site), but also permits these assays to be conducted with forms of the receptor containing only the **kinase** portions.

DETD . . . select 50 representative compounds as a "training set." Each of these 50 representative compounds was tested with respect to the **insulin** receptor. A sample believed to consist only of TER12 shown in FIG. 2A, whose fingerprint did not group and was. . .

DETD . . . activate any receptor which undergoes autophosphorylation. In general, the method comprises identifying a compound that activates a receptor containing a **kinase** portion by autophosphorylation. The method comprises contacting each member of a set of maximally diverse candidate compounds with the receptor or **kinase** portion of the receptor and detecting the presence or absence of tyrosine phosphate on the receptor or **kinase** portion. A successful candidate is identified as a member of the set wherein an increased level of tyrosine phosphate as compared to basal is detected in the receptor or **kinase** with which it was contacted.

DETD . . . it is of no consequence that TER12 and TER3938 were themselves later shown to be less active in the IR **kinase** assays than other components contained in samples of these compounds with respect to the utility of their fingerprints for identification of compounds that have IR **kinase** activity since the active contaminants are chemically similar.

DETD . . . those that are shared by several active compounds, in contrast, for example, to the compounds which do not activate the **insulin** receptor, permits the design of suitable candidates for synthesis and testing. Methods for such analysis and identification of such structural. . .

DETD [0071] Once activators, of the **insulin** receptor (or any receptor) have been identified either by screening a maximally diverse library or by using the results of. . . wherein the activator compounds, for example labeled with radioisotopes, fluorescent labels, enzyme labels, and the like, are contacted with the **insulin** receptor or the **insulin** receptor **kinase** in the presence and absence of candidate **insulin** receptor activator

compounds. The amount of label bound to the receptor or to its **kinase** portion is measured in the presence and absence of the candidate; an increased level of label binding in the absence,. . .

DETD Apparent Effect of TER12 on **Insulin** Receptor **Kinase** Autophosphorylation

DETD . . . A. This assay is a modified form of that described in Hagino, H. et al. Diabetes (1994) 43:274-280. Briefly, human **insulin** receptors (hIR) were partially purified from placental extracts or from cell line IM-9. The partially purified hIRs were captured into. . . minutes with wells coated with a monoclonal antibody to hIR. The wells were then treated with various dose levels of **insulin** and/or test compounds for 15 minutes at room temperature; ATP (10 .mu.M) was then added to permit **kinase** activity to proceed. After 60 minutes, the wells were washed, and then treated for 60 minutes with biotinylated antibody directed. . .

DETD [0074] When tested in this assay, **insulin** gave a dose response curve showing an EC.sub.50 of about 0.3 nM and a maximal activity at about 100 nM. The EC.sub.50 is similar to that obtained for binding of labeled **insulin** to various cells and tissues.

DETD . . . 100 compounds, only a sample composed mainly of TER12 (see FIG.

2A) showed apparent agonist activity. In the absence of **insulin**, 20 .mu.M of this sample stimulated autophosphorylation over five-fold (0.3 nM **insulin** stimulates phosphorylation approximately to this extent). Thus, the activity of **insulin** at approximately 0.3 nM is roughly equivalent to that shown by this sample at approximately 20 .mu.M and a component. . .

DETD [0076] In addition, the sample enhanced the ability of **insulin** to stimulate autophosphorylation. The addition of 60 .mu.M sample to hIR

contacted with 0.3 nM **insulin** resulted in an increase in phosphorylation of approximately three-fold and to the maximal level shown by **insulin** stimulation at higher concentrations. The EC.sub.50 for this effect (enhancing **insulin** stimulation) was shown in additional experiments to be approximately 20 .mu.M of sample calculated as TER12. These results were also. . .

DETD . . . activation of receptor prepared as in Paragraph A. About 20 .mu.M of sample calculated as TER12 provided 75% of maximal **insulin**-stimulated activity; it also enhanced the ability of 0.5 nM and 5.0 nM **insulin** to effect phosphorylation; 0.5 nM **insulin** alone showed 60% maximal phosphorylation; addition of 20 .mu.M of the TER12 sample increased this to 120%; in the presence of 5 nM **insulin** phosphorylation rose from 95% of maximum to 140%.

DETD [0078] C. When tested with respect to **insulin** receptor agonist activity on whole cells, i.e., on the human lymphocytic cell line IM-9, the sample containing TER12 retained its. . . to stimulate the receptor. In this assay, 2.times.10.sup.7 cells were treated with and without this sample and with and without **insulin** for 5 minutes, followed by three washes in isotonic medium to remove the sample containing TER12. The cells were then. . . Paragraph A, without the steps of incubation with ATP. After 5 minutes exposure to sample containing 20 .mu.M TER12, basal **insulin** receptor **kinase** activity was increased two-fold and **insulin** stimulated **insulin** receptor **kinase** activity was increased five-fold.

DETD [0079] D. The assay described in paragraph B was conducted by substituting, for the human **insulin** receptor, a recombinantly produced .beta. chain lacking the **insulin**-binding domain (supplied by Stratagene, Inc.). The ability of this **kinase** to phosphorylate a substrate peptide (Raytide from Oncogene Sciences) is

stimulated by TER12 at 25 .mu.M. (In addition, a known inhibitor believed to act at the ATP site on the **kinase** also inhibits this modified form of the receptor.)

DETD [0080] E. **Insulin** is able to induce the differentiation of 3T3-L1 fibroblast cells to an adipocyte-like morphology as measured by Oil Red O. . . . alone does not appear to effect differentiation; however, at a concentration of 20 .mu.M it enhances the differentiating effect of **insulin**. This activity is similar to that exhibited by pioglitazone described above. **Insulin** also enhances glucose transport in this cell line. Again, the sample alone failed to stimulate glucose transport significantly, but enhanced the ability of **insulin** to do so.

DETD . . . Yellow No. 27, showed an EC.sub.50 of 8 .mu.M in this in vitro assay; it also enhanced the activity of **insulin** in stimulating autophosphorylation of **insulin** receptor on intact IM-9 cells. In addition, a sample containing TER3935, shown in FIG. 2C, was active in the IR **kinase** assay.

DETD . . . washed with aqueous sodium carbonate, the insoluble compound shown in FIG. 2B as TER3938 was less active in the IR **kinase** assay; the aqueous layer, however, retained full activity. These results led to the conclusion that some of the activity shown. . . Component A, obtained from commercial sources, was purified by C-18 reverse-phase preparative HPLC and retained its activity in the IR **kinase** assay. Component A was subsequently demonstrated to be a minor component in samples containing both TER12 and TER3938. No Component. . .

DETD . . . commercially supplied sample, enhances glucose uptake in differentiated 3T3-L1 cells, and the activity is not dependent on the presence of **insulin**. It is, however, dependent on the activity of PI-3 **kinase**, confirming that the glucose uptake is mediated via the **insulin** signaling pathway. The ability of 16 .mu.g/ml concentrations of Component A to enhance glucose uptake at various **insulin** concentrations is shown in FIG. 4.

DETD . . . days after induction, the cells were treated with 16 .mu.g/ml of Component A in the presence of various levels of **insulin** for 30 minutes. Glucose uptake was measured using .sup.14C glucose as label. As shown, 16 .mu.g/ml of Component A alone effects uptake at approximately the level shown by 100 nM concentrations of **insulin** in the presence of this concentration of Component A.

DETD [0088] TER16998 activates the **insulin** receptor **kinase** directly, enhances autophosphorylation and substrate phosphorylation mediated through the **insulin** receptor, potentiates glucose transport and lowers blood glucose in the db/db mouse model of diabetes. These results were obtained as. . .

DETD . . . The assay described in Example 1, paragraph A, was conducted with a control lacking any additions, in the presence of **insulin** alone at 1 nM, in the presence of TER16998 at 2 .mu.M and in the presence of a combination of. . . alone is able to activate autophosphorylation of the receptor at this concentration, as well as to potentiate the effect of **insulin**.

DETD . . . assay for glucose uptake by 3T3-L1 adipocytes, described in Example 3, TER16998 produced an acute effect sensitizing the cells to **insulin**. This was inhibited, as expected, by 5 .mu.M wortmannin which inhibits PI-3 **kinase**, confirming that TER16998 exerts its effect through the **insulin**-signaling pathway. These results are shown in FIG. 7. As shown, 40 .mu.M of TERI 6998 potentiates

the effect of **insulin** at a range of concentrations.

DETD [0091] Significantly, TER16998 was not able to stimulate the phosphorylation activity of epidermal growth factor receptor in an EGF receptor **kinase** assay.

DETD [0092] The effect of TER16998, of Component A, and of **insulin** on the distribution of the Glut4 transporter in 3 T3-L1 adipocytes was determined by incubating the cells for 15 minutes with **insulin** or one of these compounds, after which the cells were fixed and stained with an anti-Glut4 antibody followed by FITC-conjugated secondary antibody. The results were visualized under a fluorescent microscope. The results showed that **insulin** and Component A produce a dramatic redistribution of Glut4 to the membrane surfaces whereas in untreated cells a diffuse pattern is obtained. TER16998 has a similar effect but less dramatic: than that of **insulin** or Component A.

DETD [0096] TER17003 was tested in the IR **kinase** assay set forth in Example 1, paragraph A, and found to be active in this assay.

CLM What is claimed is:

1. A method to modulate the **kinase** activity of **insulin** receptor which method comprises contacting said **insulin** receptor or the **kinase** portion thereof with a compound of the formula ##STR7## wherein each A is independently a proton-accepting substituent, each R is. . . linker is independently an isostere of --N.dbd.N-- or of --NHCO--; said compound provided in an amount effective to modulate said **kinase** activity.

6. A method to potentiate the **insulin** activation of **insulin** receptor which method comprises contacting said **insulin** receptor or the **kinase** portion thereof with **insulin** and with a compound of the formula ##STR10## wherein each A is independently a proton-accepting substituent; each R is independently. . . linker is independently an isostere of --N.dbd.N-- or of --NHCO--; said compound provided in an amount effective to potentiate said **insulin** activation.

11. A method to potentiate the stimulation by **insulin** of cellular glucose uptake which method comprises contacting cells displaying the **insulin** receptor with **insulin** and with a compound of the formula ##STR13## wherein each A is independently a proton-accepting substituent; each R is independently. . .

16. A method to stimulate the uptake of glucose in cells displaying the **insulin** receptor which method comprises contacting said cells with a compound of the formula ##STR16## wherein each A is independently a. . .

26. A method to identify a compound that activates a receptor containing a **kinase** portion by autophosphorylation, which method comprises contacting each member of a set of maximally diverse candidate compounds with said receptor or **kinase** portion thereof; detecting the amount of phosphotyrosine on the receptor or **kinase** portion contacted with each set member; and identifying as a successful candidate at least one member of the set wherein phosphotyrosine is detected in increased amount in the receptor or **kinase** with which it was contacted.

27. The method of claim 26 wherein said detecting of tyrosine phosphate comprises contacting said receptor or **kinase** portion with an antibody immunoreactive with tyrosine **kinase**; and detecting any complex formed between said antibody and said receptor or

kinase portion.

29. A method to design and synthesize a molecule that activates the **insulin** receptor which method comprises assessing an activator identified by the method of claim 26 or TER12, TER3938, TER3935, TER16998, TER17003 or other compound shown to activate the **kinase** activity of the **insulin** receptor for structural features which correlate with said activities; synthesizing a compound containing these structural features; and testing said compound for its ability to activate the **insulin** receptor to verify it as an activator.

30. A method to screen candidate compounds for ability to activate the **kinase** activity **insulin** receptor, which method comprises obtaining a fingerprint of each candidate with respect to a reference panel; obtaining a fingerprint of. . . identified by the method of claim 26 or TER12, TER3938, TER3935, TER16998, TER17003 or other compound shown to activate the **kinase** activity of the **insulin** receptor with respect to the same reference panel; comparing the fingerprint of each candidate with that of any of said.

. identified by the method of claim 26 or TER12, TER3938, TER3935, TER16998, TER17003 or other compound shown to activate the **kinase** activity of the **insulin** receptor; and identifying as the successful candidate a compound whose fingerprint resembles that of an activator identified by the method of claim 26 or TER12, TER3938, TER3935. TER16998, TER17003 or other compound shown to activate the **kinase** activity of the **insulin** receptor.

32. A method to identify a candidate compound which will activate the **insulin** receptor which method comprises contacting a sample containing at least the **kinase** portion of the **insulin** receptor with an activator identified by the method of claim 26 in the presence and absence of said candidate; measuring. . .
33. The method of claim 32 wherein said binding is measured by the activation of the **insulin** receptor.

36. A method to identify a candidate compound which will activate the **insulin** receptor which method comprises contacting a sample containing at least the **kinase** portion of the **insulin** receptor with an activator identified by the method of claim 29 in the presence and absence of said candidate; measuring. . .
37. The method of claim 36 wherein said binding is measured by the activation of the **insulin** receptor.

40. A method to identify a candidate compound which will activate the **insulin** receptor which method comprises contacting a sample containing at least the **kinase** portion of the **insulin** receptor with an activator identified by the method of claim 30 in the presence and absence of said candidate; measuring. . .
41. The method of claim 40 wherein said binding is measured by the activation of the **insulin** receptor.

IT **10190-68-8P**, TER 3938
(modulators of insulin receptor activity, screening, and therapeutic use)
IT **17681-50-4P**, TER 12 **210978-64-6P**, TER 16998
(modulators of insulin receptor activity, screening, and therapeutic

use)

L9 ANSWER 2 OF 7 USPATFULL
AB Modulation of the activity of the **insulin** receptor, enhancement of glucose uptake by cells, and other effects significant in the control and management of diabetes are accomplished using compounds of the formula ##STR1##

wherein each A is independently a proton-accepting substituent;

each R is independently a noninterfering substituent;

m is 0 or 1;

n is 0, 1, or 2; and

each linker is independently --NHCNHNH--, --NHCOO--, OCOO--,--CH.dbd.CH--, --CH.dbd.N--, --CH.sub.2 CH.sub.2 --, --NHCH.sub.2 --, --OCO-- or --COO--. Compounds in the genus of Formula (1) can also be used for structure activity studies to identify features responsible for the relevant activities.

AN 2001:226684 USPATFULL
TI Nonpeptide **insulin** receptor agonists
IN Sportsman, Richard, San Francisco, CA, United States
Villar, Hugo O., Newark, CA, United States
Kauvar, Lawrence M., San Francisco, CA, United States
Satyam, Apparao, Freemont, CA, United States
PA Telik, Inc., South San Francisco, CA, United States (U.S. corporation)
PI US 6329431 B1 20011211
AI US 1997-916088 19970821 (8)
RLI Continuation of Ser. No. US 1997-784855, filed on 15 Jan 1997
DT Utility
FS GRANTED
EXNAM Primary Examiner: Jones, Dwayne C.
LREP Heller Ehrman White & McAuliffe LLP
CLMN Number of Claims: 25
ECL Exemplary Claim: 1
DRWN 16 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 763
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
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SUMM . . . receptors specific for them so that the activity of the hormone is felt on designated cells exhibiting these receptors. The **insulin** receptor is present on virtually all cells and at high concentrations on the cells of the liver, skeletal muscles, and adipose tissue. Stimulation of the **insulin** receptor with **insulin** is an essential element in carbohydrate metabolism and storage.
SUMM Diabetics either lack sufficient endogenous secretion of the

insulin hormone (Type I) or have an **insulin** receptor-mediated signalling pathway that is to some degree resistant to endogenous or exogenous **insulin**, either through primary or post-translational structural changes, reduced numbers or poor coupling among signaling components (Type II). All Type I diabetics, and many Type II subjects as well, must utilize injection to obtain enhanced activity of the extant **insulin** receptors, since endogenous **insulin** can at present be replaced only with an alternative supply of **insulin** itself, previously isolated from native sources, and now recombinantly produced. While the recombinant production of **insulin** permits a less immunogenic form to be provided and assures a reliable supply of needed quantities, the necessity to administer. . . of peptides and proteins in the digestive tract. It has long been the goal to substitute for peptide ligands, including **insulin**, small molecules which are not digested and can be absorbed directly into the bloodstream. However, to date, nonpeptide substances which can exert the effect of **insulin** on its receptor have eluded discovery.

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SUMM . . . compounds, whose synthesis is straightforward, in order to conduct assays for the ability of candidate small molecules to activate the **insulin** receptor and to design these candidates. The method of identifying a primary member of this group, TER2 and of obtaining the remaining members is described below. These small molecules represent the first instance of direct agonist activity on the **insulin** receptor by a nonpeptide. Compounds identified in this way are useful in the control and management of diabetes in suitable.

SUMM Thus, the invention is directed to methods to modulate the **kinase** activity of the **insulin** receptor or the **kinase** portion thereof; to potentiate **insulin** activation of the **insulin** receptor; to potentiate glucose uptake stimulation by **insulin**; to lower blood glucose; and to stimulate glucose uptake per se in cells by use of compounds having the formula. . .

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receptor. The method comprises first obtaining a fingerprint of each candidate with respect to a reference panel and obtaining a fingerprint of TER12, TER3938, TER3935, TER16998, TER17003 or other compound shown to activate the **kinase** activity of the **insulin** receptor with respect to the same reference panel. Then the fingerprint of each candidate is compared with that of any. . .

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SUMM In still another aspect, the invention provides an alternative method to identify a candidate compound which will activate the **insulin** receptor. This method comprises contacting a sample containing at least the **kinase** portion of the **insulin** receptor with an activator identified by any of the foregoing methods in the presence and absence of said candidate.

DRWD FIG. 1 shows a schematic diagram of the **insulin** receptor and its activation by **insulin**.

DRWD FIGS. 2A-2F show the structures of several compounds relevant to the invention which activate the **insulin** receptor.

DRWD FIG. 4 shows the effect of Component A on **insulin**-induced uptake of glucose by adipocytes.

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DETD The structure of the **insulin** receptor and some aspects of its mode of action as currently understood, are illustrated in FIG. 1. The receptor consists. . . two .beta. chains contain a cross-membrane domain, the a portions are in the extracellular domain and accommodate the binding of **insulin**. The illustration in FIG. 1 shows **insulin** bound to the receptor. The .beta. subunits contain a tyrosine **kinase** activity, shown as the white inserts into the subunits and the **kinase** of one .beta. subunit effects the phosphorylation of the complementary .beta. subunit as shown, the receptor illustrated in FIG. 1. . . in its activated form when the tyrosine residues (Y) are phosphorylated. The .beta. subunits also contain ATP binding sites. The **insulin**-stimulated phosphorylation of the receptor itself is required for subsequent activity and thus demonstration of the ability of a compound to. . .

DETD . . . to methods to regulate and manage subjects with diabetes by virtue of administering compounds which affect the activity of the **insulin** receptor. Without intending to be bound by any theory, it is believed that the compounds useful in the methods of the invention act directly on the **kinase** function of the receptor and do not necessarily compete with **insulin** for binding at the **insulin**-binding site, nor do they effect activation of the receptor by a mechanism similar to that exhibited by **insulin**. The compounds of the invention are able directly to activate the **kinase** of the receptor to autophosphorylate, to potentiate the effect of **insulin** on the receptor, to activate the **kinase** function of the receptor in phosphorylating exogenous substrates, to effect the increased uptake of glucose by adipocytes and **insulin** receptor-bearing cells in general, and to lower blood

glucose levels in diabetic subjects.

DETD . . . comprises, in a preferred embodiment, contacting each member of a set of maximally diverse candidate compounds with said receptor or **kinase** portion thereof; detecting the presence or absence of tyrosine phosphate on the receptor or **kinase** portion contacted with each set member; and identifying as a successful candidate at least one member of the set wherein an increased amount of tyrosine phosphate is detected in the receptor or **kinase** with which it was contacted, relative to untreated receptor.

DETD In addition, once a compound with at least moderate ability to activate the **kinase** activity of **insulin** receptor has been identified, additional compounds can be identified by comparing the properties of the candidates with those of compounds. . . This is described in U.S. Pat. No. 5,587,293, incorporated herein by reference. Further, analysis of compounds shown to activate the **insulin** receptor **kinase** using standard structure activity analysis will result in additional compounds which behave as activators. Compounds identified as activators of the. . .

DETD The three primary methods of identification of compounds with the desired IR **kinase** modulating activity are illustrated below.

DETD The activator compounds are able to stimulate the phosphorylation catalyzed by IR **kinase** alone, i.e., to behave as agonists with respect to the receptor and/or are able to enhance the ability of **insulin** to effect phosphorylation of the receptor. Either of these effects can be considered an activation of the **insulin** receptor. Thus, by "activating" the **insulin** receptor is meant either the ability to behave as an agonist or the ability to enhance the stimulation by **insulin** or other agonists of the receptor activity. Both of these effects can be evidenced by autophosphorylation of the receptor.

DETD The compounds of the invention evidently do not interact with the receptor at the native **insulin** binding site, but rather at a site located on the **kinase** portion of the receptor. Thus, these compounds define a newly discovered activation site for this receptor. This permits not only. . . with the same site), but also permits these assays to be conducted with forms of the receptor containing only the **kinase** portions.

DETD . . . select 50 representative compounds as a "training set." Each of these 50 representative compounds was tested with respect to the **insulin** receptor. A sample believed to consist only of TER12 shown in FIG. 2A, whose fingerprint did not group and was. . .

DETD . . . activate any receptor which undergoes autophosphorylation. In general, the method comprises identifying a compound that activates a receptor containing a **kinase** portion by autophosphorylation. The method comprises contacting each member of a set of maximally diverse candidate compounds with the receptor or **kinase** portion of the receptor and detecting the presence or absence of tyrosine phosphate on the receptor or **kinase** portion. A successful candidate is identified as a member of the set wherein an increased level of tyrosine phosphate as compared to basal is detected in the receptor or **kinase** with which it was contacted.

DETD . . . is of no consequence that TERI 2 and TER3938 were themselves later shown to be less active in the IR **kinase** assays than other components contained in samples of these compounds with respect to the utility of their fingerprints for identification of compounds that

have IR **kinase** activity since the active contaminants are chemically similar.

DETD . . . those that are shared by several active compounds, in contrast, for example, to the compounds which do not activate the **insulin** receptor, permits the design of suitable candidates for synthesis and testing. Methods for such analysis and identification of such structural. . .

DETD Once activators of the **insulin** receptor (or any receptor) have been identified either by screening a maximally diverse library or by using the results of. . . wherein the activator compounds, for example labeled with radioisotopes, fluorescent labels, enzyme labels, and the like, are contacted with the **insulin** receptor or the **insulin** receptor **kinase** in the presence and absence of candidate **insulin** receptor activator compounds. The amount of label bound to the receptor or to its **kinase** portion is measured in the presence and absence of the candidate; an increased level of label binding in the absence,. . .

DETD Apparent Effect of TER12 on **Insulin** Receptor **Kinase** Autophosphorylation

DETD A. This assay is a modified form of that described in Hagino, H. et al. Diabetes (1994) 43:274-280. Briefly, human **insulin** receptors (hIR) were partially purified from placental extracts or from cell line IM-9. The partially purified hIRs were captured into. . . minutes with wells coated with a monoclonal antibody to hIR. The wells were then treated with various dose levels of **insulin** and/or test compounds for 15 minutes at room temperature; ATP (10 .mu.M) was then added to permit **kinase** activity to proceed. After 60 minutes, the wells were washed, and then treated for 60 minutes with biotinylated antibody directed. . .

DETD When tested in this assay, **insulin** gave a dose response curve showing an EC.sub.50 of about 0.3 nM and a maximal activity at about 100 nM. The EC.sub.50 is similar to that obtained for binding of labeled **insulin** to various cells and tissues.

DETD . . . 100 compounds, only a sample composed mainly of TER12 (see FIG. 2A) showed apparent agonist activity. In the absence of **insulin**, 20 .mu.M of this sample stimulated autophosphorylation over five-fold (0.3 .mu.M **insulin** stimulates phosphorylation approximately to this extent). Thus, the activity of **insulin** at approximately 0.3 nM is roughly equivalent to that shown by this sample at approximately 20 .mu.M and a component. . .

DETD In addition, the sample enhanced the ability of **insulin** to stimulate autophosphorylation. The addition of 60 .mu.M sample to hIR contacted with 0.3 nM **insulin** resulted in an increase in phosphorylation of approximately three-fold and to the maximal level shown by **insulin** stimulation at higher concentrations. The EC.sub.50 for this effect (enhancing **insulin** stimulation) was shown in additional experiments to be approximately 20 .mu.M of sample calculated as TER12. These results were also. . .

DETD . . . activation of receptor prepared as in Paragraph A. About 20 .mu.M of sample calculated as TER12 provided 75% of maximal **insulin**-stimulated activity; it also enhanced the ability of 0.5 nM and 5.0 nM **insulin** to effect phosphorylation; 0.5 nM **insulin** alone showed 60% maximal phosphorylation; addition of 20 .mu.M of the TER12 sample increased this to 120%; in the presence of 5 nM **insulin** phosphorylation rose from 95% of maximum to 140%.

DETD C. When tested with respect to **insulin** receptor agonist activity on whole cells, i.e., on the human lymphocytic cell line IM-9, the sample containing TER12 retained its. . . to stimulate the receptor. In this assay, 2.times.10.sup.7 cells were treated with and without this sample and with and without **insulin** for 5 minutes, followed by three washes in isotonic medium to remove the sample containing TER12. The cells were then. . . Paragraph A, without the steps of incubation with ATP. After 5 minutes exposure to sample containing 20 .mu.M TER12, basal **insulin** receptor **kinase** activity was increased two-fold and **insulin** stimulated **insulin** receptor **kinase** activity was increased five-fold.

DETD D. The assay described in paragraph B was conducted by substituting, for the human **insulin** receptor, a recombinantly produced .beta. chain lacking the **insulin**-binding domain (supplied by Stratagene, Inc.). The ability of this **kinase** to phosphorylate a substrate peptide (Raytide from Oncogene Sciences) is stimulated by TER12 at 25 .mu.M. (In addition, a known inhibitor believed to act at the ATP site on the **kinase** also inhibits this modified form of the receptor.)

DETD E. **Insulin** is able to induce the differentiation of 3T3-L1 fibroblast cells to an adipocyte-like morphology as measured by Oil Red O. . . alone does not appear to effect differentiation; however, at a concentration of 20 .mu.M it enhances the differentiating effect of **insulin**. This activity is similar to that exhibited by pioglitazone described above. **Insulin** also enhances glucose transport in this cell line. Again, the sample alone failed to stimulate glucose transport significantly, but enhanced the ability of **insulin** to do so.

DETD . . . Yellow No. 27, showed an EC.sub.50 of 8 .mu.M in this in vitro assay; it also enhanced the activity of **insulin** in stimulating autophosphorylation of **insulin** receptor on intact IM-9 cells. In addition, a sample containing TER3935, shown in FIG. 2C, was active in the IR **kinase** assay.

DETD . . . washed with aqueous sodium carbonate, the insoluble compound shown in FIG. 2B as TER3938 was less active in the IR **kinase** assay; the aqueous layer, however, retained full activity. These results led to the conclusion that some of the activity shown. . . Component A, obtained from commercial sources, was purified by C-18 reverse-phase preparative BPLC and retained its activity in the IR **kinase** assay. Component A was subsequently demonstrated to be a minor component in samples containing both TER12 and TER3938. No Component. . .

DETD . . . commercially supplied sample, enhances glucose uptake in differentiated 3T3-L1 cells, and the activity is not dependent on the presence of **insulin**. It is, however, dependent on the activity of PI-3 **kinase**, confirming that the glucose uptake is mediated via the **insulin** signaling pathway. The ability of 16 .mu.g/ml concentrations of Component A to enhance glucose uptake at various **insulin** concentrations is shown in FIG. 4.

DETD . . . days after induction, the cells were treated with 16 .mu.g/ml of Component A in the presence of various levels of **insulin** for 30 minutes.

DETD . . . As shown, 16 .mu.g/ml of Component A alone effects uptake at approximately the level shown by 100 mM concentrations of **insulin** in the presence of this concentration of Component A.

DETD TER16998 activates the **insulin** receptor **kinase** directly, enhances autophosphorylation and substrate phosphorylation mediated through the **insulin** receptor, potentiates glucose transport and lowers blood glucose in the db/db mouse model of diabetes.

These results were obtained as. . .

DETD The assay described in Example 1, paragraph A, was conducted with a control lacking any additions, in the presence of **insulin** alone at 1 nM, in the presence of TER16998 at 2 .mu.M, and in the presence of a combination of. . . alone is able to activate autophosphorylation of the receptor at this concentration, as well as to potentiate the effect of **insulin**.

DETD . . . assay for glucose uptake by 3T3-L1 adipocytes, described in Example 3, TER16998 produced an acute effect sensitizing the cells to **insulin**. This was inhibited, as expected, by 5 .mu.M wortmannin which inhibits PI-3 **kinase**, confirming that TER16998 exerts its effect through the **insulin**-signaling pathway. These results are shown in FIG. 7. As shown, 40 .mu.M of TER16998 potentiates the effect of **insulin** at a range of concentrations.

DETD Significantly, TER16998 was not able to stimulate the phosphorylation activity of epidermal growth factor receptor in an EGF receptor **kinase** assay.

DETD The effect of TER16998, of Component A, and of **insulin** on the distribution of the Glut4 transporter in 3 T3-L1 adipocytes was determined by incubating the cells for 15 minutes with **insulin** or one of these compounds, after which the cells were fixed and stained with an anti-Glut4 antibody followed by FITC-conjugated secondary antibody. The results were visualized under a fluorescent microscope. The results showed that **insulin** and Component A produce a dramatic redistribution of Glut4 to the membrane surfaces whereas in untreated cells a diffuse pattern is obtained. TER16998 has a similar effect but less dramatic than that of **insulin** or Component A.

DETD TER17003 was tested in the IR **kinase** assay set forth in Example 1, paragraph A, and found to be active in this assay.

CLM What is claimed is:

1. A method to modulate the **kinase** activity of **insulin** receptor which method comprises contacting said **insulin** receptor or the **kinase** portion thereof with a compound of Formula (1): ##STR7## wherein each A is independently a proton-accepting substituent; each R is. . . --CH.dbd.CH--, --CH.dbd.N--, --CH.sub.2 CH.sub.2 --, --NHCH.sub.2 --, --OCO-- or --COO--, said compound provided in an amount effective to modulate said **kinase** activity.

6. A method to potentiate the **insulin** activation of **insulin** receptor which method comprises contacting said **insulin** receptor or the **kinase** portion thereof with a compound of Formula (1): ##STR10## wherein each A is independently a proton-accepting substituent; each R is. . . --CH.dbd.CH--, --CH.dbd.N--, --CH.sub.2 CH.sub.2 --, --NHCH.sub.2 --, --OCO-- or --COO--, said compound provided in an amount effective to potentiate said **insulin** activation.

11. A method to potentiate the stimulation by **insulin** of cellular glucose uptake which method comprises contacting cells displaying the **insulin** receptor with **insulin** and with a compound of Formula (1): ##STR13## wherein each A is independently a proton-accepting substituent; each R is independently.

. .
16. A method to stimulate the uptake of glucose in cells displaying the **insulin** receptor which method comprises contacting said cells with a compound of Formula (1): ##STR16## wherein each A is independently a. . .

IT **10190-68-8P**, TER 3938
(modulators of insulin receptor activity, screening, and therapeutic use)

IT **17681-50-4P**, TER 12 **210978-64-6P**, TER 16998
(modulators of insulin receptor activity, screening, and therapeutic use)

L9 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2002 ACS

AB In type 2 diabetes, impaired **insulin** signaling leads to hyperglycemia and other metabolic abnormalities. To study a new class of antidiabetic agents, we compared two small, nonpeptide mols. that activate **insulin** receptor (IR) .beta.-subunit tyrosine **kinase** activity: Merck L 7, a direct IR agonist, and Telik's TLK 16998, an IR sensitizer. In rat hepatoma cells (HTCs) that overexpress the IR (HTC-IR), IR autophosphorylation was directly activated by L 7 in the absence of **insulin**. TLK 16998 did not directly activate IR autophosphorylation, but it enhanced IR autophosphorylation in the presence of **insulin**. Tyrosine phosphorylation of an endogenous 185-kDa IR substrate was also significantly enhanced by both Merck L 7 alone and TLK16998 plus **insulin**. Adding TLK 16998 to L 7 produced synergistic effects, further indicating that these two compds. act on the IR through sep. mechanisms. We next studied HTC-IR.DELTA.485-599 cells, which overexpress a mutant IR with a deletion in the .alpha.-subunit connecting domain that does not undergo autophosphorylation in response to **insulin** binding. L 7 was able to directly activate autophosphorylation of the deletion mutant IR in these cells, whereas TLK 16998 had no effect. Compds. were then tested in three other cell models of impaired IR function. Both TLK 16998 and Merck L 7 improved IR autophosphorylation in cells with diminished IR signaling due to either treatment with tumor necrosis factor-.alpha. or overexpression of membrane glycoprotein PC-1. However, in TPA (tetradecanoylphorbol acetate)-treated cells, TLK 16998 but not Merck L 7 was able to significantly reverse the impaired **insulin** -stimulated IR autophosphorylation. In summary, these two classes of IR activators selectively increased IR function in a variety of **insulin**-resistant cell lines.

AN 2001:735295 CAPLUS

DN 136:95887

TI Small molecule **insulin** receptor activators potentiate **insulin** action in **insulin**-resistant cells

AU Li, Ming; Youngren, Jack F.; Manchem, Vara Prasad; Kozlowski, Michael; Zhang, Bei B.; Maddux, Betty A.; Goldfine, Ira D.

CS Mount Zion Medical Center, University of California at San Francisco, San Francisco, CA, 94143-1616, USA

SO Diabetes (2001), 50(10), 2323-2328
CODEN: DIAEAZ; ISSN: 0012-1797

PB American Diabetes Association

DT Journal

LA English

RE.CNT 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Small molecule **insulin** receptor activators potentiate **insulin** action in **insulin**-resistant cells

AB In type 2 diabetes, impaired **insulin** signaling leads to hyperglycemia and other metabolic abnormalities. To study a new class of antidiabetic agents, we compared two small, nonpeptide mols. that activate **insulin** receptor (IR) .beta.-subunit tyrosine **kinase** activity: Merck L 7, a direct IR agonist, and Telik's TLK 16998, an IR sensitizer. In rat hepatoma cells (HTCs) that overexpress the IR (HTC-IR), IR autophosphorylation was directly activated by L 7 in the absence of **insulin**. TLK 16998 did not directly activate IR autophosphorylation, but it enhanced IR autophosphorylation in the presence of **insulin**. Tyrosine phosphorylation of an endogenous 185-kDa IR substrate was also significantly enhanced by both Merck L 7 alone and TLK16998 plus **insulin**. Adding TLK 16998 to L 7 produced synergistic effects, further indicating that these two compds. act on the IR through sep. mechanisms. We next studied HTC-IR.DELTA.485-599 cells, which overexpress a mutant IR with a deletion in the .alpha.-subunit connecting domain that does not undergo autophosphorylation in response to **insulin** binding. L 7 was able to directly activate autophosphorylation of the deletion mutant IR in these cells, whereas TLK 16998 had no effect. Compds. were then tested in three other cell models of impaired IR function. Both TLK 16998 and Merck L 7 improved IR autophosphorylation in cells with diminished IR signaling due to either treatment with tumor necrosis factor-.alpha. or overexpression of membrane glycoprotein PC-1. However, in TPA (tetradecanoylphorbol acetate)-treated cells, TLK 16998 but not Merck L 7 was able to significantly reverse the impaired **insulin** -stimulated IR autophosphorylation. In summary, these two classes of IR activators selectively increased IR function in a variety of **insulin**-resistant cell lines.

ST MerckL7 TLK16998 synergistic interaction antidiabetic **insulin** receptor phosphorylation

IT Antidiabetic agents
(small mol. **insulin** receptor activators potentiate **insulin** action in **insulin**-resistant cells)

IT Drug interactions
(synergistic; small mol. **insulin** receptor activators potentiate **insulin** action in **insulin**-resistant cells)

IT Phosphoproteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(tyrosine-contg., phosphorylation of **insulin** receptors; small mol. **insulin** receptor activators potentiate **insulin** action in **insulin**-resistant cells)

IT **Insulin** receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(.beta.-subunit; small mol. **insulin** receptor activators potentiate **insulin** action in **insulin**-resistant cells)

IT **210978-64-6**, TLK 16998
RL: PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(TLK 16998; small mol. **insulin** receptor activators potentiate **insulin** action in **insulin**-resistant cells)

IT 9004-10-8, **Insulin**, biological studies
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL

(Biological study); USES (Uses)
(small mol. **insulin** receptor activators potentiate **insulin** action in **insulin**-resistant cells)

IT 78860-34-1
RL: PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(small mol. **insulin** receptor activators potentiate **insulin** action in **insulin**-resistant cells)

L9 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2002 ACS

AB **Insulin** resistance, an important feature of type 2 diabetes, is manifested as attenuated **insulin** receptor (IR) signaling in response to **insulin** binding. A drug that promotes the initiation of IR signaling by enhancing IR autophosphorylation should, therefore, be useful for treating type 2 diabetes. This report describes the effect of a small mol. IR sensitizer, TLK16998, on IR signaling. This compd. activated the tyrosine **kinase** domain of the IR .beta.-subunit at concns. of 1 .mu.mol/l or less but had no effect on **insulin** binding to the IR .alpha.-subunit even at much higher concns. TLK16998 alone had no effect on IR signaling in mouse 3T3-L1 adipocytes but, at concns. as low as 3.2 .mu.mol/l, enhanced the effects of **insulin** on the phosphorylation of the IR .beta.-subunit and IR substrate 1, and on the amt. of phosphatidylinositol 3-**kinase** that co-immunopptd. with IRS-1. Phosphopeptide mapping revealed that the effect of TLK16998 on the IR was assocd. with increased tyrosine phosphorylation of the activation loop of the .beta.-subunit tyrosine **kinase** domain. TLK16998 also increased the potency of **insulin** in stimulating 2-deoxy-D-glucose uptake in 3T3-L1 adipocytes, with a detectable effect at 8 .mu.mol/l and a 10-fold increase at 40 .mu.mol/l. In contrast, only small effects were obsd. on IGF-1-stimulated 2-deoxy-D-glucose uptake. In diabetic mice, TLK16998, at a dose of 10 mg/kg, lowered blood glucose levels for up to 6 h. These results suggest, therefore, that small nonpeptide mols. that directly sensitize the IR may be useful for treating type 2 diabetes.

AN 2001:257061 CAPLUS

DN 135:71039

TI A novel small molecule that directly sensitizes the **insulin** receptor in vitro and in vivo

AU Manchem, Vara Prasad; Goldfine, Ira D.; Kohanski, Ronald A.; Cristobal, Cristina P.; Lum, Robert T.; Schow, Steven R.; Shi, Songyuan; Spevak, Wayne R.; Laborde, Edgardo; Toavs, Deborah K.; Villar, Hugo O.; Wick, Michael M.; Kozlowski, Michael R.

CS Telik, Inc., South San Francisco, CA, 94080, USA

SO Diabetes (2001), 50(4), 824-830
CODEN: DIAEAZ; ISSN: 0012-1797
PB American Diabetes Association
DT Journal
LA English

RE.CNT 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI A novel small molecule that directly sensitizes the **insulin** receptor in vitro and in vivo

AB **Insulin** resistance, an important feature of type 2 diabetes, is manifested as attenuated **insulin** receptor (IR) signaling in response to **insulin** binding. A drug that promotes the initiation of IR signaling by enhancing IR autophosphorylation should, therefore, be useful for treating type 2 diabetes. This report describes

the effect of a small mol. IR sensitizer, TLK16998, on IR signaling. This compd. activated the tyrosine **kinase** domain of the IR .beta.-subunit at concns. of 1 .mu.mol/l or less but had no effect on **insulin** binding to the IR .alpha.-subunit even at much higher concns. TLK16998 alone had no effect on IR signaling in mouse 3T3-L1 adipocytes but, at concns. as low as 3.2 .mu.mol/l, enhanced the effects of **insulin** on the phosphorylation of the IR .beta.-subunit and IR substrate 1, and on the amt. of phosphatidylinositol 3-**kinase** that co-immunopptd. with IRS-1. Phosphopeptide mapping revealed that the effect of TLK16998 on the IR was assocd. with increased tyrosine phosphorylation of the activation loop of the .beta.-subunit tyrosine **kinase** domain. TLK16998 also increased the potency of **insulin** in stimulating 2-deoxy-D-glucose uptake in 3T3-L1 adipocytes, with a detectable effect at 8 .mu.mol/l and a 10-fold increase at 40 .mu.mol/l. In contrast, only small effects were obsd. on IGF-1-stimulated 2-deoxy-D-glucose uptake. In diabetic mice, TLK16998, at a dose of 10 mg/kg, lowered blood glucose levels for up to 6 h. These results suggest, therefore, that small nonpeptide mols. that directly sensitize the IR may be useful for treating type 2 diabetes.

ST antidiabetic TLK16998 sensitize **insulin** receptor glucose; autophosphorylation tyrosine **kinase** signal pathway TLK16998

IT Transport proteins
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(GLUT-4 (glucose-transporting, 4); TLK16998 sensitizes **insulin** receptor in vitro and in vivo)

IT Phosphoproteins
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(IRS-1 (**insulin** receptor substrate 1); TLK16998 sensitizes **insulin** receptor in vitro and in vivo)

IT Antidiabetic agents
Signal transduction, biological
(TLK16998 sensitizes **insulin** receptor in vitro and in vivo)

IT **Insulin** receptors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(TLK16998 sensitizes **insulin** receptor in vitro and in vivo)

IT Phosphorylation, biological
(autophosphorylation; TLK16998 sensitizes **insulin** receptor in vitro and in vivo)

IT Diabetes mellitus
(non-**insulin**-dependent; TLK16998 sensitizes **insulin** receptor in vitro and in vivo)

IT **210978-64-6**, TER 16998
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES
(Uses)
(TLK16998 sensitizes **insulin** receptor in vitro and in vivo)

IT 80449-02-1, Tyrosine **kinase** 115926-52-8, Phosphatidylinositol 3-**kinase**
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(TLK16998 sensitizes **insulin** receptor in vitro and in vivo)

IT 50-99-7, D-Glucose, biological studies

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(blood; TLK16998 sensitizes **insulin** receptor in vitro and in vivo)

IT 9004-10-8, **Insulin**, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(resistance; TLK16998 sensitizes **insulin** receptor in vitro and in vivo)

L9 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2002 ACS

AB Methods to identify compds. which have .gtoreq.1 characteristic selected from the group consisting of a compn. that (a) modulates the **kinase** activity of **insulin** receptor; and/or (b) potentiates the **insulin** activation of **insulin** receptor; and/or (c) potentiates the stimulation by **insulin** of cellular glucose uptake; and/or (d) stimulates the uptake of glucose in cells displaying the **insulin** receptor; and/or (e) lowers blood glucose in diabetic subjects; and/or (f) stimulates IRS-1 phosphorylation; and/or (g) stimulates PI3 **kinase** activity; and/or (h) stimulates GLUT-4 translocation; are described. Successful substances having such characteristics alter the conformation of the two-lobed cytoplasmic **kinase** domain or preferentially bind sites which have been identified as modulator binding sites in the **insulin** receptor .beta. chain. Also, modulation of the activity of the **insulin** receptor, enhancement of glucose uptake by cells, and other effects significant in the control and management of diabetes are accomplished using [Ari(A)(R)mlinkeri]nAr(A)(R)m (Ar = arom. moiety; A = proton-accepting substituent; R = non-interfering substituent; m = 0-2 n = 1-6; linker = CH2, N=N, CH=CH, NHCO, NHCONH or isostere thereof; when n = 1, .gtoreq.1 Ar must comprise .gtoreq.2 fused arom. rings) (I). I can also be used for structure-activity studies to identify features responsible for the relevant activities.

AN 1998:509345 CAPLUS

DN 129:144864

TI Modulators of **insulin** receptor activity, screening, and therapeutic use

IN Kauvar, Lawrence M.; Sportsman, Richard; Villar, Hugo O.; Spevak, Wayne R.; Kohanski, Ron A.; Satyam, Apparao; Koehler, Ryan

PA Terrapin Technologies, Inc., USA

SO PCT Int. Appl., 77 pp.
CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9832017	A2	19980723	WO 1998-US801	19980115
	WO 9832017	A3	19990225		
	W: AU, BA, CA, CU, GH, GM, GW, ID, JP, LC, SL, YU, ZW				
	RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
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	US 5851988	A	19981222	US 1997-784854	19970115
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	AU 9860266	A1	19980807	AU 1998-60266	19980115
	EP 960335	A2	19991201	EP 1998-903515	19980115
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,				

IE, FI

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	US 1997-825269	A	19970327		
	US 1997-916088	A	19970821		
	WO 1998-US801	W	19980115		

OS MARPAT 129:144864

TI Modulators of **insulin** receptor activity, screening, and therapeutic use

AB Methods to identify compds. which have .gtoreq.1 characteristic selected from the group consisting of a compn. that (a) modulates the **kinase** activity of **insulin** receptor; and/or (b) potentiates the **insulin** activation of **insulin** receptor; and/or (c) potentiates the stimulation by **insulin** of cellular glucose uptake; and/or (d) stimulates the uptake of glucose in cells displaying the **insulin** receptor; and/or (e) lowers blood glucose in diabetic subjects; and/or (f) stimulates IRS-1 phosphorylation; and/or (g) stimulates PI3 **kinase** activity; and/or (h) stimulates GLUT-4 translocation; are described. Successful substances having such characteristics alter the conformation of the two-lobed cytoplasmic **kinase** domain or preferentially bind sites which have been identified as modulator binding sites in the **insulin** receptor .beta. chain. Also, modulation of the activity of the **insulin** receptor, enhancement of glucose uptake by cells, and other effects significant in the control and management of diabetes are accomplished using [Ari(A)(R)mlinkeri]nAr(A)(R)m (Ar = arom. moiety; A = proton-accepting substituent; R = non-interfering substituent; m = 0-2 n = 1-6; linker = CH2, N=N, CH=CH, NHCO, NHCONH or isostere thereof; when n = 1, .gtoreq.1 Ar must comprise .gtoreq.2 fused arom. rings) (I). I can also be used for structure-activity studies to identify features responsible for the relevant activities.

ST **insulin** receptor modulator screening antidiabetic; **kinase insulin** receptor modulator screening; glucose uptake **insulin** receptor modulator; hypoglycemic **insulin** receptor modulator; IRS1 phosphorylation **insulin** receptor modulator; GLUT4 translocation **insulin** receptor modulator; phosphoinositol **kinase** stimulation **insulin** receptor modulator; structure activity **insulin** receptor modulator design

IT Transport proteins
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(GLUT-4 (glucose-transporting, 4), translocation; modulators of **insulin** receptor activity, screening, and therapeutic use)

IT Phosphoproteins
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(IRS-1 (**insulin** receptor substrate 1), phosphorylation; modulators of **insulin** receptor activity, screening, and therapeutic use)

IT Phosphorylation, biological
(autophosphorylation; modulators of **insulin** receptor activity, screening, and therapeutic use)

IT Energy transfer
(fluorescence energy transfer; modulators of **insulin** receptor activity, screening, and therapeutic use)

IT Gene
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(for modified **insulin** receptor .beta.-chain; modulators of **insulin** receptor activity, screening, and therapeutic use)
IT Conformation
(**insulin** receptor .beta.-chain double-lobed cytoplasmic core **kinase** domain; modulators of **insulin** receptor activity, screening, and therapeutic use)
IT Mutation
(modified **insulin** receptor .beta.-chain; modulators of **insulin** receptor activity, screening, and therapeutic use)
IT Antidiabetic agents
Combinatorial library
Drug design
Drug screening
Fluorescent substances
Phosphorylation, biological
(modulators of **insulin** receptor activity, screening, and therapeutic use)
IT **Insulin** receptors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(modulators of **insulin** receptor activity, screening, and therapeutic use)
IT Antibodies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(to **insulin** receptor .beta.-chain; modulators of **insulin** receptor activity, screening, and therapeutic use)
IT Biological transport
(uptake, glucose; modulators of **insulin** receptor activity, screening, and therapeutic use)
IT 9000-83-3, ATPase 9077-69-4, Phosphoinositol **kinase**
88201-45-0, **Insulin** receptor **kinase**
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(modulators of **insulin** receptor activity, screening, and therapeutic use)
IT **10190-68-8P**, TER 3938
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PUR (Purification or recovery); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(modulators of **insulin** receptor activity, screening, and therapeutic use)
IT **17681-50-4P**, TER 12 **210978-64-6P**, TER 16998
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(modulators of **insulin** receptor activity, screening, and therapeutic use)
IT 210826-85-0 210826-86-1 210826-87-2
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(modulators of **insulin** receptor activity, screening, and therapeutic use)

IT 4156-21-2 20324-87-2 210826-90-7
RL: RCT (Reactant); RACT (Reactant or reagent)
(reaction; modulators of **insulin** receptor activity, screening, and therapeutic use)
IT 50-99-7, Glucose, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(uptake; modulators of **insulin** receptor activity, screening, and therapeutic use)

L9 ANSWER 6 OF 7 USPATFULL
AB Modulation of the activity of the **insulin** receptor, enhancement of glucose uptake by cells, and other effects significant in
the control and management of diabetes are accomplished using compounds of the formula ##STR1## wherein

each A is independently a proton-accepting substituent;

each R is independently a noninterfering substituent;

n is 0, 1, or 2; and

each linker is independently an isostere of --NHCONH-- or of --N.dbd.N--
or of --NHCO--.

Compounds in the genus of Formula (1) can also be used for structure activity studies to identify features responsible for the relevant activities.
AN 1998:159920 USPATFULL
TI Nonpeptide **insulin** receptor agonists
IN Sportsman, Richard, San Francisco, CA, United States
Villar, Hugo O., Newark, CA, United States
Kauvar, Lawrence M., San Francisco, CA, United States
Spevak, Wayne R., Albany, CA, United States
PA Terrapin Technologies, Inc., South San Francisco, CA, United States (U.S. corporation)
PI US 5851988 19981222
AI US 1997-784854 19970115 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Fitzgerald, David L.; Assistant Examiner: Pak, Michael
CLMN Number of Claims: 25
ECL Exemplary Claim: 1
DRWN 16 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 731
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI Nonpeptide **insulin** receptor agonists
AB Modulation of the activity of the **insulin** receptor, enhancement of glucose uptake by cells, and other effects significant in
the control and management of diabetes are accomplished. . .
SUMM . . . for peptide ligands that activate hormone receptors. More specifically, it concerns simple nonpeptide compounds that behave as agonists for the **insulin** receptor and enhance the effect of **insulin** on this receptor.
SUMM . . . receptors specific for them so that the activity of the hormone

is felt on designated cells exhibiting these receptors. The **insulin** receptor is present on virtually all cells and at high concentrations on the cells of the liver, skeletal muscles, and adipose tissue. Stimulation of the **insulin** receptor with **insulin** is an essential element in carbohydrate metabolism and storage.

SUMM Diabetics either lack sufficient endogenous secretion of the **insulin** hormone (Type I) or have an **insulin** receptor-mediated signalling pathway that is to some degree resistant to endogenous or exogenous **insulin**, either through primary or post-translational structural changes, reduced numbers or poor coupling among signaling components (Type II). All Type I diabetics, and many Type II subjects as well, must utilize injection to obtain enhanced activity of the extant **insulin** receptors, since endogenous **insulin** can at present be replaced only with an alternative supply of **insulin** itself, previously isolated from native sources, and now recombinantly produced. While the recombinant production of **insulin** permits a less immunogenic form to be provided and assures a reliable supply of needed quantities, the necessity to administer. . . of peptides and proteins in the digestive tract. It has long been the goal to substitute for peptide ligands, including **insulin**, small molecules which are not digested and can be absorbed directly into the bloodstream. However, to date, nonpeptide substances which can exert the effect of **insulin** on its receptor have eluded discovery.

SUMM . . . a peptide hormone. The ability of certain thiazolidinediones such as pioglitazone to enhance adipocyte differentiation by stimulating the effect of **insulin** has been described by, for example, Kletzien, R. F. et al. J Mol Pharmacol (1992) 41:393-398. These represent a class of potential antidiabetic compounds that act at an unknown site downstream from the **insulin** receptor itself and enhance the response of target tissues to **insulin**. Kobayashi, M. Diabetes (1992) 41:476-483. It is now known that most of the thiazolidinediones bind to PPAR.gamma. thus triggering certain nuclear events that may result in enhanced sensitivity of the target cells to **insulin**. However, the complete mechanism is still unresolved.

SUMM . . . that several aryl di- or polysulfonate compounds which share certain common structural features are able to effect stimulation of the **insulin** receptor to activate the autophosphorylation activity required for signal transduction. The availability of these compounds permits construction of assays and. . . comparative procedures for evaluating additional candidate compounds as well as the design and synthesis of therapeutics for primary treatment of **insulin** resistance and diabetics with the appropriate structural features.

SUMM . . . compounds, whose synthesis is straightforward, in order to conduct assays for the ability of candidate small molecules to activate the **insulin** receptor and to design these candidates. The method of identifying a primary member of this group, TER12 and of obtaining the remaining members is described below. These small molecules represent the first instance of direct agonist activity on the **insulin** receptor by a nonpeptide. Compounds identified in this way are usefull in the control and management of diabetes in suitable. . .

SUMM Thus, the invention is directed to methods to modulate the **kinase** activity of the **insulin** receptor or the **kinase** portion thereof; to potentiate **insulin**

activation of the **insulin** receptor; to potentiate glucose uptake stimulation by **insulin**; to lower blood glucose; and to stimulate glucose uptake per se in cells by use of compounds having the formula. . .

SUMM In another aspect, the invention is directed to a method to screen candidate compounds for ability to activate the **insulin** receptor. The method comprises first obtaining a fingerprint of each candidate with respect to a reference panel and obtaining a fingerprint of TER12, TER3938, TER3935, TER16998 or other compound shown to activate the **kinase** activity of the **insulin** receptor with respect to the same reference panel. Then the fingerprint of each candidate is compared with that of any. . .

SUMM In another aspect, the invention relates to a method to design and synthesize a molecule that exhibits agonist activity or **insulin** agonist stimulating activity with respect to the **insulin** receptor. This method comprises assessing an activator identified as above for structural features which correlate with said activities. Structural features. . .

SUMM In still another aspect, the invention provides an alternative method to identify a candidate compound which will activate the **insulin** receptor. This method comprises contacting a sample containing at least the **kinase** portion of the **insulin** receptor with an activator identified by any of the foregoing methods in the presence and absence of said candidate.

DRWD FIG. 1 shows a schematic diagram of the **insulin** receptor and its activation by **insulin**.

DRWD FIGS. 2A-2F show the structures of several compounds relevant to the invention which activate the **insulin** receptor. FIG. 2A shows the structure of TER12, Cibacron Brilliant Red 3BA; FIG. 2B shows the structure of TER3938, Direct. . .

DRWD FIG. 4 shows the effect of Component A on **insulin**-induced uptake of glucose by adipocytes.

DRWD FIG. 6 shows the effect of TER16998, alone and in combination with **insulin**, on autophosphorylation of the IR receptor.

DRWD FIG. 7 shows the effect of TER16998 on **insulin**-induced glucose uptake in adipocytes.

DETD The structure of the **insulin** receptor and some aspects of its mode of action as currently understood, are illustrated in FIG. 1. The receptor consists. . . two .beta. chains contain a cross-membrane domain; the .alpha. portions are in the extracellular domain and accommodate the binding of **insulin**. The illustration in FIG. 1 shows **insulin** bound to the receptor. The .beta. subunits contain a tyrosine **kinase** activity, shown as the white inserts into the subunits and the **kinase** of one .beta. subunit effects the phosphorylation of the complementary .beta. subunit as shown; the receptor illustrated in FIG. 1. . . in its activated form when the tyrosine residues (Y) are phosphorylated. The .beta. subunits also contain ATP binding sites. The **insulin**-stimulated phosphorylation of the receptor itself is required for subsequent activity and thus demonstration of the ability of a compound to. . .

DETD . . . to methods to regulate and manage subjects with diabetes by virtue of administering compounds which affect the activity of the **insulin** receptor. Without intending to be bound by any theory, it is believed that the compounds useful in the methods of the invention act directly on the **kinase** function of the receptor and do not

necessarily compete with **insulin** for binding at the **insulin**-binding site, nor do they effect activation of the receptor by a mechanism similar to that exhibited by **insulin**. The compounds of the invention are able directly to activate the **kinase** of the receptor to autophosphorylate, to potentiate the effect of **insulin** on the receptor, to activate the **kinase** function of the receptor in phosphorylating exogenous substrates, to effect the increased uptake of glucose by adipocytes and **insulin** receptor-bearing cells in general, and to lower blood glucose levels in diabetic subjects.

DETD . . . comprises, in a preferred embodiment, contacting each member of a set of maximally diverse candidate compounds with said receptor or **kinase** portion thereof; detecting the presence or absence of tyrosine phosphate on the receptor or **kinase** portion contacted with each set member; and identifying as a successful candidate at least one member of the set wherein an increased amount of tyrosine phosphate is detected in the receptor or **kinase** with which it was contacted, relative to untreated receptor.

DETD In addition, once a compound with at least moderate ability to activate the **kinase** activity of **insulin** receptor has been identified, additional compounds can be identified by comparing the properties of the candidates with those of compounds. . . This is described in U.S. Pat. No. 5,587,293, incorporated herein by reference. Further, analysis of compounds shown to activate the **insulin** receptor **kinase** using standard structure activity analysis will result in additional compounds which behave as activators. Compounds identified as activators of the. . .

DETD The three primary methods of identification of compounds with the desired IR **kinase** modulating activity are illustrated below.

DETD The activator compounds are able to stimulate the phosphorylation catalyzed by IR **kinase** alone, i.e., to behave as agonists with respect to the receptor and/or are able to enhance the ability of **insulin** to effect phosphorylation of the receptor. Either of these effects can be considered an activation of the **insulin** receptor. Thus, by "activating" the **insulin** receptor is meant either the ability to behave as an agonist or the ability to enhance the stimulation by **insulin** or other agonists of the receptor activity. Both of these effects can be evidenced by autophosphorylation of the receptor.

DETD The compounds of the invention evidently do not interact with the receptor at the native **insulin** binding site, but rather at a site located on the **kinase** portion of the receptor. Thus, these compounds define a newly discovered activation site for this receptor. This permits not only. . . with the same site), but also permits these assays to be conducted with forms of the receptor containing only the **kinase** portions.

DETD . . . select 50 representative compounds as a "training set." Each of these 50 representative compounds was tested with respect to the **insulin** receptor. A sample believed to consist only of TER12 shown in FIG. 2A, whose fingerprint did not group and was. . .

DETD . . . activate any receptor which undergoes autophosphorylation. In general, the method comprises identifying a compound that activates a receptor containing a **kinase** portion by autophosphorylation. The method comprises contacting each member of a set of maximally diverse candidate compounds with the receptor or **kinase** portion of the receptor and detecting the presence or absence of

tyrosine phosphate on the receptor or **kinase** portion. A successful candidate is identified as a member of the set wherein an increased level of tyrosine phosphate as compared to basal is detected in the receptor or **kinase** with which it was contacted.

DETD . . . it is of no consequence that TER12 and TER3938 were themselves later shown to be less active in the IR **kinase** assays than other components contained in samples of these compounds with respect to the utility of their fingerprints for identification of compounds that have IR **kinase** activity since the active contaminants are chemically similar.

DETD . . . those that are shared by several active compounds, in contrast, for example, to the compounds which do not activate the **insulin** receptor, permits the design of suitable candidates for synthesis and testing. Methods for such analysis and identification of such structural. . .

DETD Once activators of the **insulin** receptor (or any receptor) have been identified either by screening a maximally diverse library or by using the results of. . . wherein the activator compounds, for example labeled with radioisotopes, fluorescent labels, enzyme labels, and the like, are contacted with the **insulin** receptor or the **insulin** receptor **kinase** in the presence and absence of candidate **insulin** receptor activator compounds. The amount of label bound to the receptor or to its **kinase** portion is measured in the presence and absence of the candidate; an increased level of label binding in the absence,. . .

DETD Apparent Effect of TER12 on **Insulin** Receptor **Kinase** Autophosphorylation

DETD This assay is a modified form of that described in Hagino, H. et al. Diabetes (1994) 43:274-280. Briefly, human **insulin** receptors (hIR) were partially purified from placental extracts or from cell line IM-9. The partially purified hIRs were captured into. . . minutes with wells coated with a monoclonal antibody to hIR. The wells were then treated with various dose levels of **insulin** and/or test compounds for 15 minutes at room temperature; ATP (10 .mu.M) was then added to permit **kinase** activity to proceed. After 60 minutes, the wells were washed, and then treated for 60 minutes with biotinylated antibody directed. . .

DETD When tested in this assay, **insulin** gave a dose response curve showing an EC.sub.50 of about 0.3 nM and a maximal activity at about 100 nM. The EC.sub.50 is similar to that obtained for binding of labeled **insulin** to various cells and tissues.

DETD . . . 100 compounds, only a sample composed mainly of TER12 (see FIG. 2A) showed apparent agonist activity. In the absence of **insulin**, 20 .mu.M of this sample stimulated autophosphorylation over five-fold (0.3 nM **insulin** stimulates phosphorylation approximately to this extent). Thus, the activity of **insulin** at approximately 0.3 nM is roughly equivalent to that shown by this sample at approximately 20 .mu.M and a component. . .

DETD In addition, the sample enhanced the ability of **insulin** to stimulate autophosphorylation. The addition of 60 .mu.M sample to hIR contacted with 0.3 nM **insulin** resulted in an increase in phosphorylation of approximately three-fold and to the maximal level shown by **insulin** stimulation at higher concentrations. The EC.sub.50 for this effect (enhancing **insulin** stimulation) was

shown in additional experiments to be approximately 20 .mu.M of sample calculated as TER12. These results were also. . .

DETD . . . activation of receptor prepared as in paragraph A. About 20 .mu.M of sample calculated as TER12 provided 75% of maximal **insulin**-stimulated activity; it also enhanced the ability of 0.5 nM and 5.0 nM **insulin** to effect phosphorylation; 0.5 nN **insulin** alone showed 60% maximal phosphorylation; addition of 20 .mu.M of the TER12 sample increased this to 120%; in the presence of 5 nM **insulin** phosphorylation rose from 95% of maximum to 140%.

DETD When tested with respect to **insulin** receptor agonist activity on whole cells, i.e., on the human lymphocytic cell line IM-9, the sample containing TER12 retained its. . . to stimulate the receptor. In this assay, 2.times.10.sup.7 cells were treated with and without this sample and with and without **insulin** for 5 minutes, followed by three washes in isotonic medium to remove the sample containing TER12. The cells were then. . . paragraph A, without the steps of incubation with ATP. After 5 minutes exposure to sample containing 20 .mu.M TER12, basal **insulin** receptor **kinase** activity was increased two-fold and **insulin** stimulated **insulin** receptor **kinase** activity was increased five-fold.

DETD The assay described in paragraph B was conducted by substituting, for the human **insulin** receptor, a recombinantly produced .beta. chain lacking the **insulin**-binding domain (supplied by Stratagene, Inc.). The ability of this **kinase** to phosphorylate a substrate peptide (Raytide from Oncogene Sciences) is stimulated by TER12 at 25 .mu.M. (In addition, a known inhibitor believed to act at the ATP site on the **kinase** also inhibits this modified form of the receptor.)

DETD **Insulin** is able to induce the differentiation of 3T3-L1 fibroblast cells to an adipocyte-like morphology as measured by Oil Red O. . . alone does not appear to effect differentiation; however, at a concentration of 20 .mu.M it enhances the differentiating effect of **insulin**. This activity is similar to that exhibited by pioglitazone described above. **Insulin** also enhances glucose transport in this cell line. Again, the sample alone failed to stimulate glucose transport significantly, but enhanced the ability of **insulin** to do so.

DETD . . . Yellow No. 27, showed an EC.sub.50 of 8 .mu.M in this in vitro assay; it also enhanced the activity of **insulin** in stimulating autophosphorylation of **insulin** receptor on intact IM-9 cells. In addition, a sample containing TER3935, shown in FIG. 2C, was active in the IR **kinase** assay.

DETD . . . washed with aqueous sodium carbonate, the insoluble compound shown in FIG. 2B as TER3938 was less active in the IR **kinase** assay; the aqueous layer, however, retained full activity. These results led to the conclusion that some of the activity shown. . . Component A, obtained from commercial sources, was purified by C-18 reverse-phase preparative HPLC and retained its activity in the IR **kinase** assay. Component A was subsequently demonstrated to be a minor component in samples containing both TER12 and TER3938. No Component. . .

DETD . . . commercially supplied sample, enhances glucose uptake in differentiated 3T3-L1 cells, and the activity is not dependent on the presence of **insulin**. It is, however, dependent on the activity of PI-3 **kinase**, confirming that the glucose uptake is mediated

via the **insulin** signaling pathway. The ability of 16 .mu.g/ml concentrations of Component A to enhance glucose uptake at various **insulin** concentrations is shown in FIG. 4.

DETD . . . days after induction, the cells were treated with 16 .mu.g/ml of Component A in the presence of various levels of **insulin** for 30 minutes. Glucose uptake was measured using .sup.14 C glucose as label. As shown, 16 .mu.g/ml of Component A alone effects uptake at approximately the level shown by 20 .mu.M concentrations of **insulin** in the absence of this concentration of Component A.

DETD TER16998 activates the **insulin** receptor **kinase** directly, enhances autophosphorylation and substrate phosphorylation mediated through the **insulin** receptor, potentiates glucose transport and lowers blood glucose in the db/db mouse model of diabetes.

These results were obtained as. . .

DETD The assay described in Example 1, paragraph A, was conducted with a control lacking any additions, in the presence of **insulin** alone at 1 nM, in the presence of TER16998 at 2 .mu.M and in the presence of a combination of. . . alone is able to activate autophosphorylation of the receptor at this concentration, as well as to

potentiate the effect of **insulin**.

DETD . . . assay for glucose uptake by 3T3-L1 adipocytes, described in Example 3, TER16998 produced an acute effect sensitizing the cells to **insulin**. This was inhibited, as expected, by 5 .mu.M wortmannin which inhibits PI-3 **kinase**, confiring that TER16998 exerts its effect through the **insulin**-signaling pathway. These results are shown in FIG. 7. As shown, 40 .mu.M of TER16998 potentiates the effect of **insulin** at a range of concentrations.

DETD Significantly, TER16998 was not able to stimulate the phosphorylation activity of epidermal growth factor receptor in an EGF receptor **kinase** assay.

DETD The effect of TER16998, of Component A, and of **insulin** on the distribution of the Glut4 transporter in 3T3-L1 adipocytes was determined by incubating the cells for 15 minutes with **insulin** or one of these compounds, after which the cells were fixed and stained with an anti-Glut4 antibody followed by FITC-conjugated secondary antibody. The results were visualized under a fluorescent microscope. The results showed that **insulin** and Component A produce a dramatic redistribution of Glut4 to the membrane surfaces whereas in untreated cells a diffuse pattern is obtained. TER16998 has a similar effect but less dramatic than that of **insulin** or Component A.

CLM What is claimed is:

1. A method to modulate the **kinase** activity of **insulin** receptor which method comprises contacting said **insulin** receptor or the **kinase** portion thereof with a compound of the formula ##STR6## wherein each A is independently a proton-accepting substituent; each R is. . . an isostere of --NHCONH-- or of --N.dbd.N-- or of --NHCO--; said compound provided in an amount effective to modulate said **kinase** activity.

6. A method to potentiate the **insulin** activation of **insulin** receptor which method comprises contacting said **insulin** receptor or the **kinase** portion thereof with **insulin** and with a compound of the formula ##STR9## wherein each A is independently a proton-accepting substituent; each R is independently. . . an isostere of --NHCONH-- or of --N.dbd.N-- or of --NHCO--; said compound provided in an amount effective to potentiate said **insulin** activation.

11. A method to potentiate the stimulation by **insulin** of cellular glucose uptake which method comprises contacting cells displaying the **insulin** receptor with **insulin** and with a compound of the formula ##STR12## wherein each A is independently a proton-accepting substituent; each R is independently. . .

16. A method to stimulate the uptake of glucose in cells displaying the **insulin** receptor which method comprises contacting said cells with a compound of the formula ##STR15## wherein each A is independently a. . .

IT **10190-68-8P**, TER 3938
(modulators of insulin receptor activity, screening, and therapeutic use)
IT **17681-50-4P**, TER 12 **210978-64-6P**, TER 16998
(modulators of insulin receptor activity, screening, and therapeutic use)

L9 ANSWER 7 OF 7 USPATFULL
AB Modulation of the activity of the **insulin** receptor, enhancement of glucose uptake by cells, and other effects significant in the control and management of diabetes are accomplished using compounds of the formula ##STR1## wherein each Ar is independently an aromatic moiety; each A is independently a proton-accepting substituent;

each R is independently a noninterfering substituent;

m is 0 or 1;

n is 4-6; and

each linker is independently an isostere of --CH.sub.2 --, --CH.dbd.CH-- or --NCHO--. Compounds in the genus of Formula (1) can also be used for structure activity studies to identify features responsible for the relevant activities.

AN 1998:135063 USPATFULL
TI Nonpeptide **insulin** receptor agonists
IN Sportsman, Richard, San Francisco, CA, United States
Villar, Hugo O., Newark, CA, United States
Kauvar, Lawrence M., San Francisco, CA, United States
PA Terrapin Technologies, Inc., South San Francisco, CA, United States (U.S. corporation)
PI US 5830918 19981103
AI US 1997-784857 19970115 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Weddington, Kevin E.
LREP Morrison & Foerster LLP
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 14 Drawing Figure(s); 10 Drawing Page(s)
LN.CNT 672
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI Nonpeptide **insulin** receptor agonists
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SUMM . . . receptors specific for them so that the activity of the hormone is felt on designated cells exhibiting these receptors. The **insulin** receptor is present on virtually all cells and at high concentrations on the cells of the liver, skeletal muscles, and adipose tissue. Stimulation of the **insulin** receptor with **insulin** is an essential element in carbohydrate metabolism and storage.

SUMM Diabetics either lack sufficient endogenous secretion of the **insulin** hormone (Type I) or have an **insulin** receptor-mediated signaling pathway that is to some degree resistant to endogenous or exogenous **insulin**, either through primary or post-translational structural changes, reduced numbers or poor coupling among signaling components (Type II). All Type I diabetics, and many Type II subjects as well, must utilize injection to obtain enhanced activity of the extant **insulin** receptors, since endogenous **insulin** can at present be replaced only with an alternative supply of **insulin** itself, previously isolated from native sources, and now recombinantly produced. While the recombinant production of **insulin** permits a less immunogenic form to be provided and assures a reliable supply of needed quantities, the necessity to administer. . . of peptides and proteins in the digestive tract. It has long been the goal to substitute for peptide ligands, including **insulin**, small molecules which are not digested and can be absorbed directly into the bloodstream. However, to date, nonpeptide substances which can exert the effect of **insulin** on its receptor have eluded discovery.

SUMM . . . a peptide hormone, The ability of certain thiazolidinediones such as pioglitazone to enhance adipocyte differentiation by stimulating the effect of **insulin** has been described by, for example, Kletzien, R. F. et al. J Mol Pharmacol (1992) 41:393-398. These represent a class of potential antidiabetic compounds that act at an unknown site downstream from the **insulin** receptor itself and enhance the response of target tissues to **insulin**. Kobayashi, M. Diabetes (1992) 41:476-483. It is now known that most of the thiazolidinediones bind to PPAR.gamma. thus triggering certain nuclear events that may result in enhanced sensitivity of the target cells to **insulin**. However, the complete mechanism is still unresolved.

SUMM . . . that several aryl di- or polysulfonate compounds which share certain common structural features are able to effect stimulation of the **insulin** receptor to activate the autophosphorylation activity required for signal transduction. The availability of these compounds permits construction of assays and. . . comparative procedures for evaluating additional candidate compounds as well as the design and synthesis of therapeutics for primary treatment of **insulin** resistance and diabetics with the appropriate structural features.

SUMM . . . compounds, whose synthesis is straightforward, in order to conduct assays for the ability of candidate small molecules to activate the **insulin** receptor and to design these candidates. The method of identifying a primary member of this group, TER12 and of obtaining the remaining members is described below. These small molecules represent the first instance of direct agonist activity on the **insulin** receptor by a nonpeptide. Compounds identified in this

way are useful in the control and management of diabetes in suitable.

SUMM Thus, the invention is directed to methods to modulate the **kinase** activity of the **insulin** receptor or the **kinase** portion thereof; to potentiate **insulin** activation of the **insulin** receptor; to potentiate glucose uptake stimulation by **insulin**; to lower blood glucose; and to stimulate glucose uptake per se in cells by use of compounds having the formula. . .

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SUMM In another aspect, the invention relates to a method to design and synthesize a molecule that exhibits agonist activity or **insulin** agonist stimulating activity with respect to the **insulin** receptor. This method comprises assessing an activator identified as Component A structural features which correlate with said activities. Compounds containing. . .

SUMM In still another aspect, the invention provides an alternative method to identify a candidate compound which will activate the **insulin** receptor. This method comprises contacting a sample containing at least the **kinase** portion of the **insulin** receptor with an activator which is Component A in the presence and absence of said candidate.

DRWD FIG. 1 shows a schematic diagram of the **insulin** receptor and its activation by **insulin**.

DRWD FIGS. 2A-2D show the structures of several compounds relevant to the invention which activate the **insulin** receptor. FIG. 2A shows the structure of TER12, Cibacron Brilliant Red 3BA; FIG. 2B shows the structure of TER3938, Direct. . .

DRWD FIG. 4 shows the effect of Component A on **insulin**-induced uptake of glucose by adipocytes.

DRWD FIG. 6 shows the effect of TER16998, alone and in combination with **insulin**, on autophosphorylation of the IR receptor.

DRWD FIG. 7 shows the effect of TER16998 on **insulin**-induced glucose uptake in adipocytes.

DETD The structure of the **insulin** receptor and some aspects of its mode of action as currently understood, are illustrated in FIG. 1. The receptor consists. . . two .beta. chains contain a cross-membrane domain; the .alpha. portions are in the extracellular domain and accommodate the binding of **insulin**. The illustration in FIG. 1 shows **insulin** bound to the receptor. The .beta. subunits contain a tyrosine **kinase** activity, shown as the white inserts into the subunits and the **kinase** of one .beta. subunit effects the phosphorylation of the complementary .beta. subunit as shown; the receptor illustrated in FIG. 1. . . in its activated form when the tyrosine residues (Y) are phosphorylated. The .beta. subunits also contain ATP binding sites. The **insulin**-stimulated phosphorylation of the receptor itself is required for subsequent activity and thus demonstration of the ability of a compound to. . .

DETD . . . to methods to regulate and manage subjects with diabetes by virtue of administering compounds which affect the activity of the **insulin** receptor. Without intending to be bound by any theory, it is believed that the compounds useful in the methods of the invention act directly on the **kinase** function of the receptor and do not

necessarily compete with **insulin** for binding at the **insulin**-binding site, nor do they effect activation of the receptor by a mechanism similar to that exhibited by **insulin**. The compounds of the invention are able directly to activate the **kinase** of the receptor to autophosphorylate, to potentiate the effect of **insulin** on the receptor, to activate the **kinase** function of the receptor in phosphorylating exogenous substrates, to effect the increased uptake of glucose by adipocytes and **insulin** receptor-bearing cells in general, and to lower blood glucose levels in diabetic subjects.

DETD . . . comprises, in a preferred embodiment, contacting each member of a set of maximally diverse candidate compounds with said receptor or **kinase** portion thereof; detecting the presence or absence of tyrosine phosphate on the receptor or **kinase** portion contacted with each set member; and identifying as a successful candidate at least one member of the set wherein an increased amount of tyrosine phosphate is detected in the receptor or **kinase** with which it was contacted, relative to untreated receptor.

DETD In addition, once a compound with at least moderate ability to activate the **kinase** activity of **insulin** receptor has been identified, additional compounds can be identified by comparing the properties of the candidates with those of compounds. . . This is described in U.S. Pat. No. 5,587,293, incorporated herein by reference. Further, analysis of compounds shown to activate the **insulin** receptor **kinase** using standard structure activity analysis will result in additional compounds which behave as activators. Compounds identified as activators of the. . .

DETD The three primary methods of identification of compounds with the desired IR **kinase** modulating activity are illustrated below.

DETD The activator compounds are able to stimulate the phosphorylation catalyzed by IR **kinase** alone, i.e., to behave as agonists with respect to the receptor and/or are able to enhance the ability of **insulin** to effect phosphorylation of the receptor. Either of these effects can be considered an activation of the **insulin** receptor. Thus, by "activating" the **insulin** receptor is meant either the ability to behave as an agonist or the ability to enhance the stimulation by **insulin** or other agonists of the receptor activity. Both of these effects can be evidenced by autophosphorylation of the receptor.

DETD The compounds of the invention evidently do not interact with the receptor at the native **insulin** binding site, but rather at a site located on the **kinase** portion of the receptor. Thus, these compounds define a newly discovered activation site for this receptor. This permits not only. . . with the same site), but also permits these assays to be conducted with forms of the receptor containing only the **kinase** portions.

DETD . . . select 50 representative compounds as a "training set." Each of these 50 representative compounds was tested with respect to the **insulin** receptor. A sample believed to consist only of TER12 shown in FIG. 2A, whose fingerprint did not group and was. . .

DETD . . . those that are shared by several active compounds, in contrast, for example, to the compounds which do not activate the **insulin** receptor, permits the design of suitable candidates for synthesis and testing. Methods for such analysis and identification of such structural. . .

DETD Once activators of the **insulin** receptor such as Component A (or any receptor) have been identified either by screening a maximally diverse library or by. . . wherein the activator compounds, for example labeled with radioisotopes, fluorescent labels, enzyme labels, and the like, are contacted with the **insulin** receptor or the **insulin** receptor **kinase** in the presence and absence of candidate **insulin** receptor activator compounds. The amount of label bound to the receptor or to its **kinase** portion is measured in the presence and absence of the candidate; an increased level of label binding in the absence,. . .

DETD Apparent Effect of TER12 on **Insulin** Receptor **Kinase** Autophosphorylation

DETD A. This assay is a modified form of that described in Hagino, H. et al. Diabetes (1994) 43:274-280. Briefly, human **insulin** receptors (hIR) were partially purified from placental extracts or from cell line IM-9. The partially purified hIRs were captured into. . . minutes with wells coated with a monoclonal antibody to hIR. The wells were then treated with various dose levels of **insulin** and/or test compounds for 15 minutes at room temperature; ATP (10 .mu.M) was then added to permit **kinase** activity to proceed. After 60 minutes, the wells were washed, and then treated for 60 minutes with biotinylated antibody directed. . .

DETD When tested in this assay, **insulin** gave a dose response curve showing an EC.sub.50 of about 0.3 nM and a maximal activity at about 100 nM. The EC.sub.50 is similar to that obtained for binding of labeled **insulin** to various cells and tissues.

DETD . . . 100 compounds, only a sample composed mainly of TER12 (see FIG. 2A) showed apparent agonist activity. In the absence of **insulin**, 20 .mu.M of this sample stimulated autophosphorylation over five-fold (0.3 nM **insulin** stimulates phosphorylation approximately to this extent). Thus, the activity of **insulin** at approximately 0.3 nM is roughly equivalent to that shown by this sample at approximately 20 .mu.M and a component. . .

DETD In addition, the sample enhanced the ability of **insulin** to stimulate autophosphorylation. The addition of 60 .mu.M sample to hIR contacted with 0.3 nM **insulin** resulted in an increase in phosphorylation of approximately three-fold and to the maximal level shown by **insulin** stimulation at higher concentrations. The EC.sub.50 for this effect (enhancing **insulin** stimulation) was shown in additional experiments to be approximately 20 .mu.M of sample calculated as TER12. These results were also. . .

DETD . . . activation of receptor prepared as in Paragraph A. About 20 .mu.M of sample calculated as TER12 provided 75% of maximal **insulin**-stimulated activity; it also enhanced the ability of 0.5 nM and 5.0 nM **insulin** to effect phosphorylation; 0.5 nM **insulin** alone showed 60% maximal phosphorylation; addition of 20 .mu.M of the TER12 sample increased this to 120%; in the presence of 5 nM **insulin** phosphorylation rose from 95% of maximum to 140%.

DETD C. When tested with respect to **insulin** receptor agonist activity on whole cells, i.e., on the human lymphocytic cell line IM-9, the sample containing TER12 retained its. . . to stimulate the receptor. In this assay, 2.times.10.sup.7 cells were treated with and without this sample and with and without **insulin** for 5 minutes, followed by three washes in isotonic medium to remove the sample containing TER12. The cells were then. . . Paragraph A, without the steps of incubation with ATP. After 5 minutes exposure to

sample containing 20 .mu.M TER12, basal **insulin** receptor **kinase** activity was increased two-fold and **insulin** stimulated **insulin** receptor **kinase** activity was increased five-fold.

DETD D. The assay described in paragraph B was conducted by substituting, for the human **insulin** receptor, a recombinantly produced .beta. chain lacking the **insulin**-binding domain (supplied by Stratagene, Inc.). The ability of this **kinase** to phosphorylate a substrate peptide (Raytide from Oncogene Sciences) is stimulated by TER12 at 25 .mu.M. (In addition, a known inhibitor believed to act at the ATP site on the **kinase** also inhibits this modified form of the receptor.)

DETD E. **Insulin** is able to induce the differentiation of 3T3-L1 fibroblast cells to an adipocyte-like morphology as measured by Oil Red O. . . . alone does not appear to effect differentiation; however, at a concentration of 20 .mu.M it enhances the differentiating effect of **insulin**. This activity is similar to that exhibited by pioglitazone described above. **Insulin** also enhances glucose transport in this cell line. Again, the sample alone failed to stimulate glucose transport significantly, but enhanced the ability of **insulin** to do so.

DETD . . . Yellow No. 27, showed an EC.sub.50 of 8 .mu.M in this in vitro assay; it also enhanced the activity of **insulin** in stimulating autophosphorylation of **insulin** receptor on intact IM-9 cells. In addition, a sample containing TER3935, shown in FIG. 2C, was active in the IR **kinase** assay.

DETD . . . washed with aqueous sodium carbonate, the insoluble compound shown in FIG. 2B as TER3938 was less active in the IR **kinase** assay; the aqueous layer, however, retained full activity. These results led to the conclusion that some of the activity shown. . . Component A, obtained from commercial sources, was purified by C-18 reverse-phase preparative HPLC and retained its activity in the IR **kinase** assay. Component A was subsequently demonstrated to be a minor component in samples containing both TER12 and TER3938. No Component. . .

DETD . . . commercially supplied sample, enhances glucose uptake in differentiated 3T3-L1 cells, and the activity is not dependent on the presence of **insulin**. It is, however, dependent on the activity of PI-3 **kinase**, confirming that the glucose uptake is mediated via the **insulin** signaling pathway. The ability of 16 .mu.g/ml concentrations of Component A to enhance glucose uptake at various **insulin** concentrations is shown in FIG. 4.

DETD . . . days after induction, the cells were treated with 16 .mu.g/ml of Component A in the presence of various levels of **insulin** for 30 minutes.

DETD . . . As shown, 16 .mu.g/ml of Component A alone effects uptake at approximately the level shown by 100 nM concentrations of **insulin** in the presence of this concentration of Component A.

DETD TER16998 activates the **insulin** receptor **kinase** directly, enhances autophosphorylation and substrate phosphorylation mediated through the **insulin** receptor, potentiates glucose transport and lowers blood glucose in the db/db mouse model of diabetes. These results were obtained as. . .

DETD The assay described in Example 1, paragraph A, was conducted with a control lacking any additions, in the presence of **insulin**

alone at 1 nM, in the presence of TER16998 at 2 .mu.M, and in the presence of a combination of. . . alone is able to activate autophosphorylation of the receptor at this concentration, as well as to potentiate the effect of **insulin**.

DETD . . . assay for glucose uptake by 3T3-L1 adipocytes, described in Example 3, TER16998 produced an acute effect sensitizing the cells to **insulin**. This was inhibited, as expected, by 5 .mu.M wortmannin which inhibits PI-3 **kinase**, confirming that TER16998 exerts its effect through the **insulin**-signaling pathway. These results are shown in FIG. 7. As shown, 40 .mu.M of TER16998 potentiates the effect of **insulin** at a range of concentrations.

DETD Significantly, TER16998 was not able to stimulate the phosphorylation activity of epidermal growth factor receptor in an EGF receptor **kinase** assay.

DETD The effect of TER16998 , of Component A, and of **insulin** on the distribution of the Glut4 transporter in 3T3-L1 adipocytes was determined by incubating the cells for 15 minutes with **insulin** or one of these compounds, after which the cells were fixed and stained with an anti-Glut4 antibody followed by FITC-conjugated secondary antibody. The results were visualized under a fluorescent microscope. The results showed that **insulin** and Component A produce a dramatic redistribution of Glut4 to the membrane surfaces whereas in untreated cells a diffuse pattern is obtained. TER16998 has a similar effect but less dramatic than that of **insulin** or Component A.

DETD As set forth above, the polymer of the formula ##STR9## is active in the **insulin** receptor **kinase** assay described above and exhibits the ability to potentiate **insulin** activation and glucose uptake.

CLM What is claimed is:

1. A method to modulate the **kinase** activity of **insulin** receptor which method comprises contacting said **insulin** receptor or the **kinase** portion thereof with a compound of the formula ##STR10## wherein each Ar is independently an aromatic moiety, each A is. . .

6. A method to potentiate the **insulin** activation of **insulin** receptor which method comprises contacting said **insulin** receptor or the **kinase** portion thereof with **insulin** and with a compound of the formula ##STR12## wherein each Ar is independently an aromatic moiety; each A is independently.

. . is independently an isostere of --CH.sub.2 --, --CH.dbd.CH-- or --NCHO--; said compound provided in an amount effective to potentiate said **insulin** activation.

IT **10190-68-8P**, TER 3938
(modulators of insulin receptor activity, screening, and therapeutic use)

IT **17681-50-4P**, TER 12 **210978-64-6P**, TER 16998
(modulators of insulin receptor activity, screening, and therapeutic use)